CORPUS CALLOSUM MORPHOLOGY IN RETINOPATHY-POSITIVE AND RETINOPATHY-NEGATIVE MALAWIAN CHILDREN DIAGNOSED WITH CEREBRAL MALARIA

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ABSTRACT

CORPUS CALLOSUM MORPHOLOGY IN RETINOPATHY-POSITIVE AND RETINOPATHY-NEGATIVE MALAWIAN CHILDREN DIAGNOSED WITH CEREBRAL MALARIA

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The current study investigated corpus callosum (CC) morphology in retinopathy-positive and retinopathy-negative pediatric survivors of cerebral malaria (CM). Participants were 20 children who were admitted to the Queen Elizabeth Central Hospital in Blantyre, Malawi, and satisfied the clinical case definition of CM. There were two groups: retinopathy-positive CM (n = 16) and retinopathy-negative CM (n = 4). Analysis of covariance and multivariate analysis of covariance were conducted and effect sizes were calculated on the area measurements to determine whether overall brain area, CC area, and five segments of the CC (i.e., genu, body, midbody, isthmus, and splenium) differed as a function of retinopathy status. No significant group differences emerged in any of the analyses. However, a large effect size was noted in overall brain area and a moderate effect size was detected in total CC area. For the five CC segments, a moderate effect size was detected for the genu, body, and midbody, and a large effect size was found for the splenium. The effect sizes indicate that these regions may be smaller in the retinopathy-positive group. The results suggest the retinopathy-positive group may have lost white matter in the CC, particularly in the splenium, and perhaps elsewhere in the brain. Research indicates attention deficits frequently occur in pediatric survivors of CM. This study is consistent with previous research Attention-Deficit/Hyperactivity Disorder, suggesting the CC, particularly the splenium, may be especially important in attentional processes.

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Cerebral malaria (CM) is a prevailing cause of illness and often death. It is estimated that CM affects more than 200 million people in Africa yearly and results in one million deaths annually (Snow, Craig, & Marsh, 1999). Cerebral malaria is particularly devastating in children, with an estimated case fatality rate of 19% (Murphy & Breman, 2001). A CM diagnosis is rare in adults living in malarial endemic regions, as they tend to acquire immunity; non-immune adults, however, are quite susceptible (World Health Organization, 2000).

While the investigation of pediatric CM is one of medical interest, pediatric CM is also an illness of clinical interest and concern. Neurological deficits following CM, such as paresis and ataxia, typically resolve within six months post-discharge from the hospital (van Hensbroek, Palmer, Jaffar, Schneider, & Kwiatkowski, 1997). Neurocognitive deficits, however, are common among pediatric survivors of CM and have been documented to be more persistent in nature (e.g., Carter et al., 2005b). Neurocognitive deficits in this population commonly occur in the areas of working memory and attention (Boivin et al., 2007). Birbeck and colleagues (2010) commented on the similarities in the neurocognitive profiles of pediatric survivors of retinopathy-positive CM and disruptive behavior disorders in children, specifically Attention-Deficit/Hyperactivity Disorder (ADHD). These researchers noted that 11% of the pediatric survivors of retinopathy-positive CM displayed symptoms of ADHD. Additionally, neuroimaging research in both pediatric populations reveals similarities in brain structure. For example, white matter abnormalities including corpus callosum abnormalities appear in both populations. Given the apparent link between pediatric survivors of CM and ADHD symptomatology, further investigation of the brain the structure of pediatric survivors of CM,

particularly the corpus callosum, is necessary. Continued investigation of brain structure is essential to further elucidate the pathogenesis of the disease, as well as to inform future intervention and treatment options. The following sections will begin by outlining the infection phase of the illness and theories on the pathogenesis of CM, diagnostic techniques, and treatment of CM. The extant neuropsychological and neuroimaging studies on pediatric CM will then be discussed.

Infection

Humans can be infected with five species of malaria: *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae*, *Plasmodium knowlesi* and *Plasmodium falciparum* (Cox-Singh et al., 2008). *Plasmodium falciparum*, or *P falciparum*, is the species most commonly associated with severe and complicated malaria. The life cycle of *P falciparum* begins with sporozoites, the transmissible form of the parasite. The sporozoites develop within the mosquito and are later transferred to humans when the mosquito bites the human host. Within 30 minutes the sporozoites travel to the liver. Inside the liver the parasite matures into a schizont, the next developmental phase of the asexual reproductive life cycle. A merozoite is formed after the division of the schizont. The merozoites are later released to invade the erythrocytes (red blood cells) of the host. The parasitized erythrocytes bind to receptors found on the endothelial cells of blood vessels. This process occurs when the parasite produces a ligand, a protein that binds to a receptor on the erythrocyte. The ligand that has been the most studied in *P falciparum* infections is the *P falciparum* erythrocyte membrane protein (PfEMP-1). The parasite sends the PfEMP-1 to the erythrocyte membrane to adhesive nodules called knobs (Adams, Brown, & Turner, 2002).

The process of merozoites infecting erythrocytes is referred to as invasion and marks the beginning of the erythrocytic phase of the cycle. This process occurs many times. The

merozoites residing in the erythrocytes are initially in ring form and later develop into trophozoites, which develop into schizonts. Merozoites are released at the time of schizont rupture, when the erythrocytes are destroyed. The merozoites then invade other red blood cells. When the schizonts rupture, other toxins are also released. This results in an initial symptom of fever noted in all forms of malarial illness. Common symptoms in *P falciparum* infections are fever, headache, nausea, diarrhea, and vomiting. If the symptoms go untreated, severe malaria may result. Severe malaria can take many forms; cerebral malaria, hypoglycemia, acidosis, and severe anemia are all potential complications (WHO, 2000). The earliest symptom is often fever in children diagnosed with CM. Following fever, frequent symptoms include refusal to eat or drink, vomiting, and cough. However, while the infection phase of CM has been well-documented, the pathogenesis of CM is still largely uncertain.

Pathogenesis of CM

In reviewing the literature, it appears that three hypotheses on the pathogenesis of CM are currently under primary investigation. The first hypothesis implicates tumor necrosis factor (TNF) in the development of the disease. The second hypothesis involves the investigation of the potential role of the blood brain barrier (BBB). Lastly, the third hypothesis suggests sequestration of parasitized erythrocytes in the microvasculature of the brain may be involved in the pathogenesis of CM. Each of these hypotheses will be examined in turn, in the sections that follow.

Tumor necrosis factor. Tumor necrosis factor (TNF) has been implicated in a breakdown of the BBB. Tumor necrosis factor and interleukin are both subsumed under the category of cytokines, or small proteins that mediate the body's immune responses. In high quantities, cytokines can be toxic to the central nervous system (CNS) of a child diagnosed with

CM. Increased levels of TNF have been noted in children diagnosed with CM relative to children diagnosed with a "mild disease" (Karunaweera, Grau, Gamage, Carter, & Mendis, 1992). One study reported that during paroxysm, tumor necrosis factor levels in CM patients are lower than those in humans infected with *Plasmodium vivax*, in which there are no neurological symptoms (Karunaweera et al., 1992).

Blood-brain barrier. A breakdown of the blood-brain barrier (BBB) has also been implicated in the pathogenesis of CM. The BBB is composed of endothelial cells, astrocyte projections known as astrocytic feet, and a basement membrane. The BBB restricts the exchange of substances from the blood into brain tissue. Studies using murine models have demonstrated a breakdown of the BBB (Thumwood, Hunt, Clark, & Cowden, 1988). In a post-mortem examination completed by Brown and colleagues (2001) on Malawian children diagnosed with CM, the researchers discovered an activation of cerebral endothelial cells as well as a disruption of intercellular junctions following CM. A small increase in the albumin index was discovered. This suggests a minor breakdown of the BBB occurred, although the authors report this breakdown was not sufficient to fully account for the cerebral edema noted in the children.

Animal model research also suggests a breakdown of the BBB. Murine models of fatal CM demonstrate two stages of a BBB breakdown. The first occurs three days following infection, in which a small but focal breakdown of the BBB occurs. Seven days after infection, a widespread breakdown of the BBB takes place. The plasma first escapes into the perivascular space, the area around the blood vessels, while an up-regulation of endothelial adhesion molecules, proteins found on the cell surface, is triggered by an immune response. An increase in the BBB permeability soon follows (Adams et al., 2002).

Sequestration. Cerebral edema has been observed to frequently occur in pediatric cases of CM (Newton et al., 1994). Sequestration, or the binding of the parasitized erythrocytes to the vessels, has been proposed as one potential cause of cerebral edema and cerebral edema may play a role in the pathogenesis of coma. Other cited possible causes of cerebral edema are increased intracranial pressure, changes in blood flow, cellular hypoxia, and some evidence suggests increased permeability of the blood brain barrier (Adams et al., 2002). Additionally, brain swelling has been documented in African children and at least some of this swelling may be attributed to intravascular sequestration of parasitized erythrocytes (Newton et al., 1994). Nonetheless, there is currently an incomplete understanding of the pathogenesis of CM. Despite an imperfect understanding of the development of CM, diagnosis is a necessary step of the treatment of CM. The diagnosis of pediatric CM is largely determined by the presence of *P falciparum* on a blood smear and coma.

To summarize, pediatric CM is a particularly devastating cause of illness in children. It is known that CM is a potential outcome following the bite of a mosquito infected with the parasite *P falciparum,* but the pathogenesis of CM is not yet completely understood. Cytokines, the blood brain barrier, and the sequestration of parasitized erythrocytes have been implicated in the pathogenesis of CM but further investigation is required (Adams et al., 2002).

Diagnosis

Cerebral malaria is defined by the World Health Organization (WHO) as the presence of *P falciparum* on a blood smear, coma as exemplified by a Glasgow Coma Scale score ≤ 8 (or Blantyre Coma Scale score ≤ 2), with no other known cause for coma. Table 1 provides a description of the Glasgow and Blantyre Coma Scales. The GCS was developed as a measure of consciousness and is generally utilized during the diagnostic process for traumatic brain injury.

The Blantyre Coma Scale was developed specifically for the assessment of children and is commonly utilized as a measure of severity of CM (Newton, et al., 1997).

The diagnosis of CM is made difficult by the knowledge that parasitemia is not specific to malaria disease. It is important to note that the presence of parasitemia does imply infection, but does not necessarily imply malarial illness. Thus, parasitemia is necessary but not sufficient to warrant a diagnosis of malaria illness. Consequently, at this point in time, it is not possible to causally link the presence of parasites to the patient's symptoms. In malaria-endemic regions, asymptomatic parasitemia is quite common and has been reported at approximately 40-70% (Mwangi, Ross, Snow, & Marsh, 2005). The diagnostic criteria to date for CM are not specific to severe malaria. Recently, however, a new technique has been implemented in the diagnosis of pediatric CM, which evaluates the appearance of the retina.

New diagnostic technique. *P falciparum* infections in children may result in any of the following four complications: cerebral malaria, respiratory distress, severe anemia, and hypoglycemia (World Health Organization, 2000). These four complications represent the most common complications and each may present with similar symptoms, but it is possible to develop any of these four syndromes without being infected with malaria. Diagnostically, however, the difficulty lies not with identifying parasitemic children, but distinguishing which syndromes are due to malaria and which are not. Because a number of syndromes present with similar symptomatology, it is challenging to make a correct diagnosis. The WHO definition does not in and of itself exclude other possible diagnoses. As a result, misdiagnosis is a common occurrence. It is therefore important that additional diagnostic tools with empirically demonstrated sensitivity and specificity be utilized. One such tool is direct and indirect ophthalmoscopy. One study detailed abnormal retinal findings referred to as malaria retinopathy

in children diagnosed with CM (Beare, Taylor, Harding, Lewallen, & Molyneux, 2006). The retina is developmentally an element of the central nervous system and allows for an in vivo investigation of infected microvasculature, the portion of the circulatory system referring to the small vessels (Beare et al., 2006).

Four retinal findings have been characterized: retinal whitening, vessel changes, hemorrhages, and papilledema. Only three of these retinal findings, however, constitute the features of malarial retinopathy: retinal whitening, vessel changes, and hemorrhages, as papilledema alone is not indicative of malaria (Lewallen et al., 2008). Retinal whitening results in scattered whitening of the retina. Vessel changes result in a color change in the retinal vessels to a pale orange or white color. Hemorrhages generally appear white-centered and are intra-retinal. Papilledema is the swelling of the optic disk and is often a sign of raised intracranial pressure. Research suggests papilledema and retinal hemorrhages are not noted only in CM; although, white-centered hemorrhages are not noted frequently in other conditions. Vessel changes and the specific pattern of retinal whitening, however, have yet to be characterized in a disease outside of malaria (Lewallen et al., 2008). Thus, if papilledema is noted with no signs of retinal whitening, vessel changes, or hemorrhages, an alternative diagnosis should be considered. These features have been observed in children in Malawi, Kenya, and Gambia (Beare et al., 2004a). Malarial retinopathy is now considered to be one of the best diagnostic features of CM, helping physicians distinguish between malarial and non-malarial coma (Beare et al., 2006; Taylor et al., 2004). See Figure 1 for pictures of each of the four retinal findings.

Beare and colleagues (2004a) discovered that children with retinopathy had greater than average parasite density, were more likely to have respiratory distress, and were less likely to have repeated or prolonged convulsions than those without retinopathy. There was no

statistically significant relationship between those with retinopathy who later developed neurological sequelae and those who did not have any neurological sequelae. Retinopathy was observed in a greater percentage of children who later died. Of the children with retinopathy who survived, the resolution of the coma was significantly longer as compared to children who did not have retinopathy. Additionally, the severity of the retinal signs was found to be correlated with death and coma resolution time.

In addition, papilledema and retinal hemorrhages were differentially related to death. Beare and colleagues (2004a) suggest that these retinal abnormalities are the result of two distinct processes. Papilledema and retinal hemorrhages were found to be independent predictors of death (Beare et al., 2004a). Cortical blindness does not appear to be correlated to raised intracranial pressure as indicated by papilledema. Additionally, the retinal changes associated with malarial retinopathy do not seem to impact visual acuity after one month (Beare, Sourthern, Kayira, Taylor, & Harding, 2004b).

To date, the majority of studies have not included retinopathy as a diagnostic criterion. Instead the clinical definition of CM, or the WHO definition, requires the presence of *P falciparum* on a blood smear, GCS or BCS core of ≤ 8 or ≤ 2, respectively, and no other cause for coma. Taylor et al. (2004) demonstrated malarial retinopathy is the only clinical feature that distinguished between CM and non-cerebral malaria cases. This study conducted autopsies on patients diagnosed with clinically defined CM (n = 31) and patients with non-malarial causes of coma (n = 11). It was determined that 23% (n = 7) of cases were misdiagnosed with CM, despite the fact that each of these cases fulfilled the WHO definition of CM. Examination of these patients failed to reveal parasite sequestration. Further histological examination revealed that three of the patients died from Reye's syndrome, arteriovenous malformation, and hepatic

necrosis, respectively. The remaining four patients died from severe anemia, pneumonia, and meningitis. However, when these children were diagnosed with CM, malarial retinopathy was not a recognized indicator of CM. Histopathological study revealed malarial retinopathy to be a key diagnostic feature of CM and the authors suggest the absence of malarial retinopathy is a strong indicator that CM is not the most appropriate diagnosis.

In one post-mortem investigation, ten ophthalmologists over a period of 10 years performed funduscopic examinations on the same patient as a technique for determining fatal CM. The ten ophthalmologists collectively achieved a sensitivity of 95% and specificity of 90% (Beare et al., 2006). When the WHO criteria were applied to this group of children, a specificity rate of 61% was achieved.

In summary, the WHO diagnostic criteria are not highly specific to CM; but, a new diagnostic sign, malarial retinopathy, has demonstrated high specificity and sensitivity to CM (Beare et al., 2006). This technique has improved the diagnosis of CM and medical treatments are available once a diagnosis has been made.

Medical Treatment

When treatment can be provided for CM, the standard procedure is intravenous or intramuscular quinine (Aceng, Byarugaba, & Tumwine, 2005; Whitty, 2005). Artemisinin treatments are also available and can be administered orally or via rectal or intramuscular administration. A rectal artemether has been suggested to be just as effective as intravenous quinine, appears to be well-tolerated, and was found to be a safe alternative to intramuscular quinine (Aceng et al., 2005; Barennes, Balima-Koussobue, Nargot, Charpentier, & Pussard, 2006).

A recent study examining parenteral artesunate and parenteral quinine treatment for severe falciparum malaria in African children under the age of 15 indicated that parenteral artesunate should be considered the preferred treatment for severe malaria (Dondorp et al., 2010). The authors discovered that while the incidence of neurological sequelae was not significantly different between the two treatment groups, those treated with artesunate were significantly less likely to develop a coma or demonstrate a deterioration of the coma score and convulsions and post-treatment hypoglycemia were also observed significantly less frequently in the artesunate group. The artesunate treatment was reported to be well-tolerated and did not have serious negative effects. Overall, this study demonstrated that artesunate treatment is superior to parenteral quinine.

Another study determined that the time to parasite clearance was significantly shorter in Malawian children diagnosed with CM treated by intramuscular artemether compared to those treated by intravenous quinine (Taylor et al., 1993). A more recent randomized clinical trial examined the efficacy of chloroquine versus sulfadoxine-pyrimethamine in the treatment of uncomplicated *P falciparum* malaria in children in Malawi (Laufer et al., 2006). Despite having been replaced by sulfadoxine-pyrimethamine in Malawi in 1993, this study discovered that choloroquine is again an efficacious treatment for malaria. Even when children receive expert care, however, approximately 15-20% of children die (Taylor et al., 2004; White, 1998).

Neurological and neuropsychological sequelae are frequent in children with CM even when appropriate treatment has been administered. Common neurological sequelae include ataxia, convulsions, paresis, and visual and hearing difficulties (van Hensbroek et al., 1997). Neuropsychological deficits in the areas of working memory and attention are most frequently documented (Boivin et al., 2007). These impairments are highly akin to those defined in

children diagnosed with ADHD. The following sections will summarize the relevant neuropsychological and ADHD literature.

Neuropsychology of CM

While most children diagnosed with CM no longer have frank neurological deficits (e.g., paresis and ataxia) six months following discharge from the hospital (van Hensbroek et al., 1997), the same is not true of neurocognitive deficits. Cognitive impairments have been reported in 11-28% of pediatric survivors of CM (Boivin et al., 2007) and are typically in the areas of attention, working memory, and language (Boivin et al., 2007, Carter et al., 2005b). Thus, cognitive impairment in children following CM is more common and generally more widespread than neurologic sequelae.

Boivin and colleagues (2007) investigated the neurocognitive sequelae in Ugandan children aged five – twelve years. Forty-four children diagnosed with CM were tested along with 54 children diagnosed with uncomplicated malaria and 89 healthy community children. Three assessments were administered: The Kaufman Assessment Battery for Children (K-ABC), the visual form of the Test of Variables of Attention (TOVA), and the tactual performance test (TPT). These tasks assess cognitive and memory abilities, attention, and tactile perception, respectively. It was discovered that 21.4% of the children had cognitive deficits in the areas of working memory, attention, and learning three months following their discharge from the hospital. At a six month follow-up, these children demonstrated even greater impairment. The children diagnosed with CM were 3.7 times more likely to develop a cognitive deficit in one or more domains of functioning, relative to the healthy community children. This pattern of continued cognitive decline suggests a significant number of children are likely to lose previously learned skills, as well as have difficulties learning new cognitive skills. A follow-up

study completed by John et al. (2008) discovered that 26.3% of children diagnosed with CM had at least one cognitive deficit two years following the child's discharge from the hospital. Additionally, the pediatric survivors of CM were at a 3.67-fold increased risk for developing a cognitive deficit in working memory, attention, or learning as compared to healthy community controls. Boivin (2002) evaluated 29 Senegalese children diagnosed with CM and 29 aged-matched mild malaria group. The K-ABC and the TOVA were administered to assess cognition, memory, and attention. It was determined that the CM group performed significantly lower on the K-ABC than the 'mild' malaria group. On the TOVA, the CM group committed significantly more omission errors than the mild malaria group. It was also determined that length of coma positively correlated with the number of omission errors committed.

In another neuropsychological study of pediatric survivors of CM, Carter and colleagues (2005a) assessed Kenyan children six – nine years of age diagnosed with CM and malaria with complicated seizures (M/S) in the areas of cognition, motor, speech and language, and hearing and vision. The authors determined the pediatric survivors of CM performed significantly lower than the M/S group on tasks language ability (i.e., higher level language, vocabulary, pragmatics, and non-verbal functioning). These studies provide further evidence for the long-term and extensive nature of the cognitive sequelae of CM. Carter and colleagues (2005b) discovered that cognitive impairments can persist up to nine years following recovery from CM. The results indicated that 24% of the CM and M/S children were impaired in at least one domain. While these two studies demonstrated that developmental impairments were noted in pediatric survivors of CM particularly in language skills, attention deficits were not assessed. Nonetheless, given that attention deficits are commonly reported in children diagnosed with CM, it is critical that such outcomes are assessed.

In contrast, one study failed to find evidence of neuropsychological sequelae following CM when the children did not suffer from neurological sequelae (Muntendam, Jaffar, Bleichrodt, & van Hendsbroek, 1996). This study evaluated the children using five cognitive tasks that assessed perception, reasoning, learning, and memory and seven sensory motor tasks that assessed visual motor coordination, balance, manual speed, and finger/hand/arm dexterity. A potential explanation for this incongruent finding is the absence of pure measures of attention from the assessment battery. A common neurocognitive impairment in pediatric survivors of CM is inattention. Had an attention measure been administered, the results may have been more congruous with the literature.

A number of risk factors associated with the development of cognitive impairments have been corroborated. A risk factor for later motor impairment was linked to multiple seizures. History of seizures, hypoglycemia, being under three years of age, increased intracranial pressure at admission and prolonged coma were found to be risk factors for speech and language deficits. Moreover, this study found that prolonged coma and prior seizure episodes were risk factors for the development of cognitive impairment (Idro et al., 2006). The later risk factors regarding extended duration of coma and multiple seizure episodes were supported by Boivin et al. (2007) as well. Holding et al. (1999) found that coma, hypoglycemia, seizures, and the absence of hyperpyrexia are four key signs in predicting cognitive impairment. Overall, developmental delays, particularly for language skills were discovered in children with positive retinopathy cerebral malaria (Boivin et al., 2011).

A common neurocognitive deficit among children who survived CM is inattention (e.g., Boivin et al., 2007). As these children look akin to children diagnosed with ADHD, the investigation of the potential correlates of these two disorders has caught the attention of

researchers. Boivin et al. (2011) evaluated 83 Malawian children diagnosed with retinopathy-positive CM and 95 age-matched control children. The Malawi Developmental Assessment Test (MDAT), an assessment of gross and fine motor control, language, and social skills, was administered to determine if a developmental delay was present. Also, the Achenbach Child Behavior Checklist (CBCL), a child psychiatric screening test, was administered to evaluate psychiatric symptoms of the child. Internalizing and externalizing symptoms, including depression, anxiety, and aggression were evaluated and no significant group differences were found. However, on the MDAT language domain, the retinopathy-positive group demonstrated a significantly greater possibility of a developmental delay. It is important to note that this study was among the first to assess retinopathy-positive cases of pediatric CM. As such, it is likely that the literature to date has evaluated both true and false cases of CM, suggesting the outcomes of these studies have been slightly confounded. This study represents an important first step in rectifying that confound.

To summarize, pediatric survivors of CM often exhibit neurological sequelae; however, these deficits typically resolve within six months following discharge from the hospital (van Hensbroek et al., 1997). Neurocognitive deficits, on the other hand, appear to occur in the areas of working memory and attention and have been documented to persist and even worsen over time (Boivin et al., 2002; Boivin et al., 2007; Carter et al., 2005a; Carter et al., 2005b; John et al., 2008). Research has also been conducted on the brain structure of patients diagnosed with CM.

Neuroimaging in CM

While studies linking the neuropsychological assessment of children diagnosed with CM to ADHD symptomatology have been completed, few neuroimaging studies have been completed with this population. Numerous neuroimaging studies have been performed with

children diagnosed with ADHD, however. Of those, many neuroimaging studies have investigated corpus callosum morphology because this structure allows information to be transferred between the hemispheres. Children diagnosed with ADHD demonstrate a slower response rate to stimuli, suggesting structures involved in the transference of information may be implicated in the disorder (Semrud-Clikeman et al., 1994). Hynd and colleagues (1991) revealed, after morphometric analysis of the corpus callosum, that children with ADHD had smaller corpus callosi overall, but particularly in the genu, isthmus, and splenium. A second study examined the area of the corpus callosum in children with ADHD who exhibited overactivity symptomatology. It was discovered that these children had significantly smaller splenial regions as compared to typically developing children (Semrud-Clikeman et al., 1994). The authors suggest smaller splenial regions may be correlated with the commonly observed attention difficulties observed in this population. Neuroimaging findings in children diagnosed with CM, however, are scarce and inconsistent.

Beyond case studies of adults, there are six neuroimaging studies on pediatric CM. Of these pediatric neuroimaging studies, two are case studies and a third study includes only one child patient; the remaining two patients in the study were adults (Cordoliani et al., 1998; Gamanagatti & Kandpal, 2006; Saavedra-Lozano, Booth, Weprin, & Ramilo, 2001). The other three neuroimaging studies completed computed tomography (CT) scans on children diagnosed with CM; one study examined eight children and the other two studies examined 14 children (Newton et al., 1994; Potchen et al., 2010; Ngoungo et al., 2006). Although the base rate is high, CM occurs largely in developing regions where appropriate medical care is largely unavailable (Birbeck, 2004). The neuroimaging technology necessary to conduct clinical and research scans is largely nonexistent as well. Consequently, little is known about the structural and functional

composition of the brain of children with CM. Of the available research, a few similarities among findings are noted, but most results have yet to be corroborated. The main findings of these studies implicated white matter, edema, and corpus callosum abnormalities.

White matter and edema. White matter abnormalities have been noted in a number of studies (Cascio et al., 2006; Cordoliani et al., 1998; Lecours et al., 2001; Patankar et al., 2002). Hyperintense areas of white matter were found in two adult patients and cortical infarcts were noted in one child diagnosed with CM (Cordoliani et al., 1998). Hyperintensities in the occipital cortex, and bilateral periventricular white matter and thalami were discovered in adult patients (Yadav, Sharma, Kumar, & Kumar, 2008). Patankar et al. (2002) linked neuroimaging and postmortem findings with clinical data in patients diagnosed with CM. The autopsies revealed cerebellar white matter hypoattenuation in five of the 21 patients. In addition, diffuse cerebral edema was noted in eight of the 21 patients. Newton et al. (1994) also noted diffuse cerebral edema in six of the 14 children who underwent CT scans. The authors also reported that CT scans of four of the 14 children demonstrated a decrease of grey and white matter, which appeared consistent with diffuse ischemic or hypoglycemic insult. Additionally, in a case study of a seven month-old male, CT results showed cerebellar edema and obstructive hydrocephalus (Saavedra-Lozano, Booth, Weprin, & Ramilo, 2001). Potchen and colleagues (2010) completed CT scans on children diagnosed with CM with prolonged coma. The authors found diffuse cerebral edema, with and without brainstem involvement and the infarction of large vessels. In a neuroimaging study completed on adults, the authors report that the MRI images for most of the patients appeared normal unless viewed in relation to other scans. Here, swelling of the brain during the acute phase of CM became apparent. Overall, only two patients showed evidence of

cerebral edema. Additionally, swelling of the brainstem and hydrocephalus were not noted in the patients (Looareesuwan, et al., 1995).

Ngoungou and colleagues (2006) report disparate CT results from children diagnosed with CM. Of the eight children that were scanned, five exhibited abnormalities. Diffuse atrophy was noted in one child. Asymmetrical enlargement of the ventricles was noted in another. A third child demonstrated atrophy in one hemisphere following bacterial meningitis. The remaining two children both showed necrosis of the pallidi.

Corpus callosum. A thinning of the corpus callosum was noted in children with protein-energy malnutrition during infancy (Lecours et al., 1998) and in children with developmental delay (Cascio et al., 2006). Bilateral symmetric hyperintensities in the centrum semiovale were also noted in a magnetic resonance (MR) study of a 13 year-old girl (Gamanagatti & Kandpal, 2006). These abnormalities noted in the centrum semiovale suggest possible cytotoxic edema, which is consistent with the cerebral edema noted in pediatric CM cases. Cordoliani et al. (1998) discovered white matter hyperintensities in the centrum semiovale and a lesion in the splenium in one of the three patients examined. In the MR study conducted by Cordoliani and colleagues (1998), neuropsychological testing determined deficits in memorization tasks. One week following the initial MR examination, the hyperintensities in the centrum semiovale had disappeared and the lesion in the splenium of the corpus callosum had decreased slightly. The noted deficits in memorization resolved five months later; however, the lesion of the splenium remained the same. A second study also noted a transient lesion in the splenium of the corpus callosum in three patients with epilepsy (Polster et al., 2001). Lesions in the splenium of the corpus callosum were noted in adult patients with cerebral malaria who presented with altered sensorium (Yadav et al., 2008).

To summarize, the neuroimaging research to date is sparse, particularly concerning pediatric CM. The common themes at present include white matter lesions, including abnormalities in the corpus callosum.

Conclusions

Cerebral malaria commonly afflicts children, particularly in the region with the most intense transmission of the causative agent, *P falciparum*. Despite the fact that CM affects millions of children and adults, little is currently known about the pathogenesis of CM. Research, particularly with animal models, has implicated cytokines, NO, and a slight breakdown in the BBB. Diagnosis of CM is often complicated as 'incidental parasitemias' are common in this population. The presence of parasitemia is itself insufficient to infer a causal relationship between symptom presentation and the presence of parasites. Consequently, misdiagnoses can occur. Recently however, a new diagnostic indicator of CM, malarial retinopathy, was identified and has demonstrated an increase in diagnostic specificity (Beare et al., 2006). Research studies concentrating on the neuropsychological deficits following pediatric CM are beginning to be completed. Deficits in the areas of working memory, attention, and language are among those most frequently documented (Boivin, et al., 2007; Carter et al., 2005a). Currently, few neuropsychological long-term follow-up studies have been completed. Of those that have, neuropsychological deficits have been noted as long as nine years following recovery from CM and have been demonstrated to increase over time (Boivin et al., 2007; Carter et al., 2005b). Additional long-term follow-up studies should be completed to determine the extent of the noted neuropsychological deficits.

The inattention and working memory deficits found in survivors of CM parallel those noted in ADHD. Numerous neuroimaging studies have been completed on children diagnosed

with ADHD. Neuroimaging studies that have investigated corpus callosum morphology have discovered that children diagnosed with ADHD have smaller overall corpus callosi, but specifically in the genu and splenium (e.g., Hynd et al., 1991; Semrud-Clikeman et al., 1994). Few neuroimaging studies, outside of case studies, have been completed on children diagnosed with CM. Of the available research, few findings appear to be consistent across studies. However, white matter abnormalities, cerebral edema, and structural changes in the corpus callosum have been noted. Additionally, only one neuroimaging study has been completed with retinopathy-positive CM. As previously discussed, misdiagnoses can occur in pediatric CM cases, but malarial retinopathy has demonstrated improved diagnostic sensitivity and specificity (Beare et al., 2006). Moreover, the inclusion of retinopathy-positive cases enhances the likelihood that true CM cases are being evaluated. Thus, future research should address pediatric cases of retinopathy-positive CM.

The Present Study

The purpose of this study was to investigate corpus callosum morphology in children diagnosed with retinopathy-positive and retinopathy-negative CM from Malawi, Africa. Little is known about the structure of the brain of children diagnosed with retinopathy-positive CM and the distinction between retinopathy-positive and retinopathy-negative pediatric CM has yet to be evaluated. To date, only one pediatric neuroimaging study has been completed with children diagnosed with retinopathy-positive CM. The current study was the second pediatric CM neuroimaging study to investigate retinopathy-positive. As discussed, pediatric survivors of CM demonstrate neurocognitive deficits similar to those noted in children diagnosed with ADHD (e.g., inattention, working memory). Neuroimaging studies completed with children diagnosed with ADHD have examined the corpus callosum because this structure is implicated in the

transference of information from one hemisphere to the other. Children diagnosed with ADHD demonstrate difficulty responding to stimuli quickly, which suggests the children's ability to transfer incoming information may be compromised. As pediatric survivors of CM exhibit signs of ADHD, an examination of the corpus callosum in these children was warranted. Regions of the corpus callosum of particular interest were the genu and the splenium, as these two regions have been implicated in sustained attention in children diagnosed with ADHD (e.g., Hynd et al., 1991; Semrud-Clikeman et al., 1994).

Corpus callosum morphology in retinopathy-positive and retinopathy-negative children was investigated. It was hypothesized that retinopathy-positive children would have smaller overall corpus callosum area as compared to the retinopathy-negative children. It was also hypothesized that the genu and the splenium would be significantly smaller in the retinopathy-positive children, as compared to the retinopathy-negative children. Additionally, gender differences were explored within the retinopathy-positive group, as there are no documented studies examining potential gender differences in the structure of the brain in children in Africa and none with survivors of CM.

Methods

Participants

Participants were 20 children, 11 males and nine females. The retinopathy-positive group included 16 children and the retinopathy-negative group included four children. Of the 20 patients, the average age was 48.75 months (*SD* = 24.93), with a range of 16 months to 9 years. The mean coma score at admission was 1.50 (*SD* = 0. 51). The mean length of coma was 44.14 hours (*SD* = 24.28). Six of the children, though they did not die, never fully regained consciousness (i.e., not seeing, hearing, or talking), thus length of coma could not be determined

for these children. The average parasitemia count upon admission was 199971.40 (*SD* = 291856). Of the children admitted to the Paediatric Research Ward (see below), the geometric means of parasitemia in the retinopathy-positive group was (*M* = 42273) and retinopathy-negative group was (*M* = 21128). See Table 2 for a complete account of patient demographics for the retinopathy-positive and negative groups. The participants were admitted to the Paediatric Research Ward, Department of Pediatrics, Queen Elizabeth Central Hospital, Blantyre, Malawi and treated for cerebral malaria. Queen Elizabeth Central Hospital cares for a population of 533,000 and serves as a teaching center for the only medical school in Malawi. Cases of complicated malaria are referred for care at no cost. This study is part of a larger study of the pathogenesis of fatal malaria.

Participant criteria. Cerebral malaria was defined in patients as the presence of *P falciparum* parasitemia, an acute onset of coma, with a score of two or less on the Blantyre coma scale (see Table 1) for a minimum of four hours following admission. No clinical or laboratory evidence was present for an alternative cause of coma, including hypoglycemia, meningitis, or postictal state following a seizure. All children were treated with intravenous quinine and fluid; anticonvulsants, antipyretics, and glucose were administered as needed. To determine retinopathy status, one physician examined each child by direct and indirect ophthalmoscopy following pupil dilation using tropicamide 1% and phenylephrine 2.5%. Upon admission and every day afterward, ophthalmoscopy was performed in accordance with patient cooperation and depth of coma. Retinal findings were recorded on standardized charts and scored for severity. The most advanced eye findings for each child were used and that information was relayed to the investigator. Children with chronic malnutrition were excluded from the study.

Image Acquisition

A certified technician located at the Magnetic Resonance Imaging Centre, Queen Elizabeth Central Hospital in Blantyre, Malawi, ran all scans. The images were read by an on-site, licensed radiologist trained by the Radiology Department at Michigan State University (MSU). All scans selected for this study were free from gross abnormalities. A sagittal two-dimensional T_1-weighted spoiled gradient echo pulse sequence (3D-SPGR) was performed on each subject on a .35 permanent magnet GE Signa Ovation J, with the following parameters: Repetition time (TR) = 24.0 ms, echo time (TE) = 4.9 ms – 5.1 ms, flip angle = 90°, FOV = 26 cm, matrix = 160 x 128, slice thickness = 1.5 mm/0.0sp. MRI files were received from Malawi via the MSU satellite downlink. The imaging data was transferred to the Department of Radiology at MSU and were subsequently transferred to a DVD for morphometric analysis at the Center for Neurodevelopmental Study.

Loss of scans. A total of 70 scans were acquired; however, fifty scans could not be used. This was a retrospective study, as the scans were acquired prior to the design of the current study. Thirty-six were not suitable for the specific analyses, as an SPGR series was not acquired; eleven scans were uninterpretable due to image artifacts; one scan was from a child who was not a contemporary study patient and two participants were not CM patients. As a result, 20 scans were analyzed.

Image Analysis

Alignment. The T_1-weighted images were transferred to the local Cognitive Imaging Research Center (CIRC) server and were later processed using Analysis of Functional NeuroImages (AFNI; Cox, 1996). To account for variance in head orientation during scanning, a procedure termed anterior callosal-posterior callosal (AC-PC) alignment was used to align the

brains along the anterior and posterior commissures. First, both the superior and posterior edges of the anterior commissure were identified and marked. Next, the inferior edge of the posterior commissure was marked. Lastly, two additional midsagittal points were marked. Using these five marks, AFNI then rotated the brain along the x-y-z axis in order to align the brain to a consistent orientation. All subsequent area measurements were completed using a personal computer (PC) version of AFNI (Cox, 1996).

Midsagittal slice identification. Before the corpus callosal (CC) and supratentorial area measurements were completed, the midsagittal plane was first carefully identified for each participant by a single investigator blind to patient diagnosis. The midsagittal plane was defined as the plane in which the fornix, thalamus, and cerebellum were most clearly visible. The midsagittal plane was then secondarily positive through careful visual inspection of the coronal and axial plane to ensure that the selected midsagittal plane was centered between the left and right hemispheres. Next, one-third of the brains were randomly selected to establish reliability by a second independent rater also blind to patient diagnosis. Interrater agreement was 1.00. All subsequent area measurements were completed on the identified midsagittal plane.

Corpus callosum area. The midsagittal area analysis of the CC was completed using the AFNI plug-in module "Draw Dataset" to identify the boundaries of the CC. The CC was defined as the higher-density band of fibers above the lateral ventricle and thalamus and below the cingulate gyrus in the midsagittal plane, similar to the definition used by Fine, Semrud-Clikeman, Keith, Stapleton, and Hynd (2007). Using the DrawDataset module, the area defined as the CC was "drawn" onto the overlay using a stylus and a drawing tablet. All voxels within the identified CC were set to a value of "1". Once the area of the CC was drawn for each patient, the AFNI plug-in module "3Ddump98" was used to determine the number of voxels assigned to

a value of one. One-third of the brains were randomly selected and re-measured by the author and intra-rater reliability was .989. See Figure 2 for a depiction of the CC area measurement.

Next, an automated program utilizing the x-y-z coordinates in AFNI was used to divide the corpus callosum into five equal regions. These regions, in order from most anterior to most posterior are the genu, body, midbody, isthmus, and splenium. When the anterior-posterior lengths were not evenly divisible by five, 100 segmentation iterations with random placement of remainders were performed. The calculated average number of voxels for each segment was then imported into SPSS and used in later analyses. This method has also been used in a study investigating the CC (Fine et al., 2007). See Figure 3 for an illustration of the CC segmentation.

Supratentorial area. Due to the wide age range, a supratentorial area measurement was completed for each participant in order to control for brain size. The supratentorial area, defined as the cerebrum without the cerebellum, was measured to determine intracranial area. It does not include the skull or meninges. The AFNI DrawDataset module was again used to draw onto the overlay using a stylus and a drawing tablet. All voxels identified as the intracranial area were assigned the value "1". Once the supratentorial area was drawn for each patient, the AFNI module 3Ddump98 was used to determine the number of voxels assigned the value of "1", thus producing the supratentorial area measurement. See Figure 4 for an illustration of the supratentorial area measurement. After completing the intracranial area measurements for each patient, one-third of the brains were again randomly selected and re-measured by the author, resulting in an intrarater reliability of .993.

Results

Power and Statistical Analyses

Power. The power to detect significant results was significantly reduced due to a limited sample size (N = 20). Nonetheless, the following statistical analyses will provide pilot data on this population and effect sizes rather than formal statistical analyses will be emphasized (Cohen, 1988).

Statistical analyses. First, a bivariate correlation was conducted examining the relationship between the five segments of the CC (i.e., genu, body, midbody, isthmus, and splenium), total CC area, supratentorial area, and age. The results indicated that each of the five CC segments was significantly correlated with one another and total CC area ($p < .001$) as expected. The five CC segments were not significantly correlated with the supratentorial area, which was not expected. Additionally, age was not significantly correlated with the 5 CC segments, overall CC area, or supratentorial area. Since, the correlation between age and supratentorial area approached significance (p = .090), a conservative approach was used and it was decided that age would be used as a covariate in the following analyses. It is particularly important to control for age, as the brain size increases until around the age of 12. See Table 3 for correlation values. Next, to examine whether overall brain size differed between the retinopathy-positive and negative groups, an exploratory one-way ANCOVA was conducted. No significant group differences were found on brain size, $F(1,17) = 3.47$, *n.s.* The effect size was large ($d = 1.11$), indicating a considerable difference in brain size due to retinopathy status, such that the retinopathy-positive group ($M = 9096.13$, $SD = 527.36$) was found to have smaller brain size than the retinopathy-negative group ($M = 9699.50$, $SD = 557.25$).

A one-way ANCOVA was conducted to determine whether total CC area differed between the retinopathy-positive and negative groups and no significant group differences were found on overall CC area, $F(1,17) = 0.92$, *n.s.* A moderate effect size was detected ($d = .63$),

which indicates a modest difference in CC area exists, such that the retinopathy-negative group had larger overall CC (M = 642.75, SD = 98.89) than the retinopathy-positive group (M = 580.06, SD = 99.95). As a large effect size was detected for supratentorial area measurement, suggesting the retinopathy-positive group may have a smaller overall brain area, a CC-to-brain size ratio was calculated for each participant. An ANOVA was then conducted with the calculated CC-to-brain size ratio and similar findings were found, $F(1,17) = .082$, *n.s.*, $d = .23$.

A 2 (group) x 5 (CC measure) Wilks Lambda MANCOVA with age as a covariate was conducted to determine whether the CC segments differed as a function of retinopathy status. Likely due to the small sample size, the results revealed no significant group effect, $F(1,17) = .31$, *n.s.* for CC segments. The effect of the covariate was also not significant, $F(1,17) = .19$, *n.s.* To more fully understand possible differences due to retinopathy status, effect sizes were calculated for each of the five segments of the corpus callosum to determine the magnitude of the relationship between the two groups. A small effect size was found for the isthmus ($d = .34$), moderate effect sizes were found for the genu ($d = .45$), body ($d = .56$), and midbody ($d = .55$), and a large effect size was found for the splenium ($d = .89$). Moreover, these effect sizes indicate that each of the five segments of the CC were smaller in the retinopathy-positive group. See Table 4.

Gender. Exploratory analyses were also performed to investigate whether total brain area and total CC area differ as a function of gender in the retinopathy-positive group. Such analyses were not conducted with the retinopathy-negative group due to small numbers. A one-way ANCOVA, with age as a covariate was first performed to determine if overall brain area differed between the retinopathy-positive males (n = 9) and females (n = 7) and a significant group difference was found indicating that males had a larger brain (M = 9320.00, SD = 523.79)

size than females (M = 8808.29, SD = 397.00), $F(1,13) = 4.70$, $p = .049$. The effect size was large ($d = 1.40$), indicating a considerable difference in brain size between brain males and females. A second one-way ANCOVA was conducted to determine if overall CC area differed between the retinopathy-positive males and females, but no significant group difference was found, $F(1,13) = .298$, *n.s.*, $d = .28$. See Table 5. Despite the previous insignificant result, a 2 (group) x 5 (CC segment) MANCOVA, with age as a covariate, was performed to determine if the five CC segments differed as a function of gender. As predicted, no significant group effect was detected, $F(1,13) = .431$, *n.s.* Effect sizes were calculated: genu ($d = .11$), body ($d = .52$), midbody ($d = .36$), isthmus ($d = .32$), splenium ($d = .10$).

Discussion

Cerebral malaria is a common cause of illness and often death in children in sub-Saharan Africa, resulting in an estimated case fatality rate of 19% (Snow et al., 1999). A new diagnostic method, malarial retinopathy, has been documented to improve the diagnostic specificity and sensitivity of the WHO (Beare et al., 2006; Taylor et al., 2004). As this is a new diagnostic technique, few studies, including neuroimaging and neurocognitive studies, have used this diagnostic method in conjunction with the WHO criteria to determine patient eligibility. Thus, much of the already limited literature on pediatric CM may have inadvertently included non-CM cases, which confounds the results in both the neurocognitive and neuroimaging literature.

Few neuroimaging studies have been completed with pediatric survivors of CM. Review of the available literature on pediatric survivors of CM and children diagnosed with ADHD, however, reveals that white matter abnormalities have been documented in both populations (e.g., Hynd et al., 1991; Patankar et al., 2002; Semrud-Clikeman et al., 1994; Yadav et al., 2008). A study investigating adult patients diagnosed with CM noted lesions in the splenium of the CC

(Yadav et al., 2008). The neuroimaging research has yet to investigate whether structural differences exist between retinopathy-positive and retinopathy-negative cases of pediatric CM. Consequently, the current study sought to investigate CC morphology in pediatric cases of retinopathy-positive and retinopathy-negative CM.

Findings from the study provide some support the first hypothesis that children in the retinopathy-positive group would have smaller overall CC area relative to the retinopathy-negative children. Partial support was also found for the hypothesis that the genu and the splenium would be significantly smaller in the retinopathy-positive children, as compared to the retinopathy-negative children. The following sections will discuss the results of the proposed hypotheses, as well as exploratory analyses conducted on gender in the retinopathy-positive group. Next, the results of the completed analyses will be discussed in light of the available research. Finally, the clinical implications and limitations of the current study will be discussed and suggestions for future research will be proposed.

Major Findings

Prior to examining the proposed hypotheses, an examination of whether total brain area differed between the retinopathy-positive and negative groups was completed. The results yielded no significant group differences. Nonetheless, the large effect size detected indicates a sizeable difference in brain size and suggests that the retinopathy-positive group may have a smaller brain size compared to the retinopathy-negative participants. This finding was not predicted, as diffuse cerebral edema is frequently noted in CM (e.g., Potchen et al., 2008). As a result, it was expected that the retinopathy-positive group would have larger overall brain area, due to the commonly documented widespread swelling of the brain. Age did not significantly differ between the two groups, suggesting that variance in brain development did not account for

the large effect size. It is possible that the white matter is compromised in retinopathy-positive children which resulted in smaller brain size. The current literature on CM indicates that white matter abnormalities, including abnormalities in the CC, frequently occur (e.g., Patankar et al., 2002; Yadav et al., 2008).

Some support was found for the first hypothesis that the children in the retinopathy-positive group would have smaller overall CC area compared to the retinopathy-negative group. Significant results were not detected from the main analyses; however, this finding may be attributed to a lack of power to detect significant main effects as a result of the small sample size. Therefore, an effect size was also calculated and a moderate effect size was detected, suggesting that the retinopathy-positive children may have smaller overall CC area. Thus, consistent with the literature previously mentioned, the results suggest that white abnormalities, particularly a reduction in white matter in the CC may occur in the retinopathy-positive children.

The second hypothesis that two segments of the CC, the genu and the splenium, would be significantly smaller in the retinopathy-positive group was partially supported. The results of the main analyses did not yield significant results; however, this finding may, again, be attributed to a lack of power. Consequently, effect sizes were calculated for each of the five segments and a trend providing some support for the hypotheses was found. A moderate effect size was detected for the genu, as well as the body and midbody, indicating that these regions were likely smaller in the retinopathy-positive group. While it was hypothesized that the genu would be significantly smaller in the retinopathy-positive group as compared to the retinopathy-negative group, this finding suggests a trend in the data providing some support for the hypothesis. A small effect size was observed for the isthmus of the CC; but, specific hypotheses were not generated for this region of the CC, so this result was not surprising. Finally, a large effect size

was detected for the splenium, indicating that this region of the CC was smaller in the retinopathy-positive group. This finding will be discussed in more detail in light of the available research in the section below.

Splenium. Regarding previous findings on the splenium in the neuroimaging literature, three studies examining adult patients diagnosed with CM and a fourth examining adult patients diagnosed with epilepsy have discovered transient lesions in the splenium (Hantson et al., 2010; Polster et al., 2001; Yadav et al., 2008). Additionally, the neuroimaging literature on children diagnosed with ADHD has found abnormalities in the splenium of the CC (Hynd et al., 1991; Semrud-Clikeman et al., 1994). It is possible that the splenium is important in many aspects of neurocognitive functioning, particularly in attention. As a result, a reduction in white matter may be associated with attentional processes and may therefore be linked to the deficits in attention noted in pediatric survivors of CM.

Posner and colleagues (1990) have proposed that the attentional networks in brain may be divided into three networks. The researchers argue that the attention system in the brain is structurally distinct from data processing systems in the brain; a network of anatomical areas of the brain are responsible for carrying out attention; and, the specific regions involved in attention have different functions. Thus, using this model and previous research implicating the CC in ADHD, it is possible that the posterior regions of the CC may play a critical role in attention networks in the brain. Research has demonstrated that pediatric survivors of CM frequently exhibit attention deficits (e.g., Boivin et al., 2007). Further research has demonstrated similarities between retinopathy-positive pediatric CM and ADHD, such that 11% of children diagnosed with CM in one study exhibited symptoms of ADHD (Birbeck et al., 2010). Additionally, the literature on children diagnosed with ADHD in developed countries suggests

that the splenium of the CC may be associated with the observed attention deficits (e.g., Hynd et al., 1991; Semrud-Clikeman et al., 1994). Thus, the results of this study, consistent with previous research on individuals in developed countries, suggest it is likely that the posterior regions of the CC, specifically the splenium, are implicated in attentional processes and are therefore related to the neurocognitive outcomes of attention deficits in pediatric survivors of CM.

Exploratory Analyses on Gender

Due to the limited sample size, particularly within the retinopathy-negative group, exploratory analyses were conducted on the retinopathy-positive group to examine potential gender differences. Previous work has found that men generally have larger brains compared to women (e.g., Ankney, 1992). There are no documented studies looking at this finding in children in Africa and none with survivors of CM. To that end, it was decided to explore possible gender differences. It was found that the retinopathy-positive CM males had larger brains than females. This finding is consistent with previous research which established that, even when controlling for body size, males have larger brains than females (e.g., Ankney, 1992). The current study was one of the first to possibly document this finding in African children. It is important to note, however, that the children included in this study were all diagnosed with CM; thus, this finding may not be readily extrapolated to the population at large.

An investigation of overall CC area was then conducted to determine if gender differences were present, but no significant group difference was detected. While neuroimaging studies regarding specific gender differences in the CC have not been conducted on individuals diagnosed with CM, a large body of literature exists on this topic in control participants residing in developed countries. The extant research on gender differences in the CC are highly

inconsistent, with some researchers finding that females tend to have a larger and more bulbous splenium than males and others failing to replicate these findings (De Lacoste-Utamsing & Holloway, 1982; Oppenheim, Benjamin, Lee, Nass & Gazzaniga, 1987). More recent research indicates that brain volume is the main contributor to overall CC size (Jancke & Steinmetz, 2003). Jancke and Steinmetz (2003) suggest that because the CC-to-brain size relationship follows a geometric rule, larger brains will be correlated with smaller CC-to-brain size ratios. As females typically have smaller brains, it follows that they will have larger CC ratios. Consequently, it was predicted that females would have larger overall CC areas; however, the data in the current study did not support this prediction. As previously discussed, it is possible that the white matter is compromised in pediatric survivors of CM and may be associated with the neurocognitive deficits noted in these children. Future research should continue to examine this possibility, as well as whether gender differences exist in specific regions of the CC, with a larger sample size.

Limitations

The main limitation of the current study was the limited sample size, particularly with regard to the retinopathy-negative group. The small sample likely reduced the power in the completed analyses, thus decreasing the likelihood that group differences could be detected. Thus, the results of the current study might best be understood as pilot data for future studies, such that the current study may provide information on what is to be expected with a larger sample. In addition, the sample of children included in the study varied widely in age. The ages of the study patients ranged from sixteen months – nine years. Thus, the results may have been influenced by the wide variance in age in the sample. Also, it is unclear why the brains of the retinopathy-positive children have smaller brains than the retinopathy-negative children, as grey

and white matter analyses were not completed to determine if either tissue is specifically affected. It may be the case that the brains of the retinopathy-positive children have always been smaller than the retinopathy-negative children; but this hypothesis should be explored in future studies (*personal communication with Dr. Michael Potchen, 2011*).

Clinical Implications

Current research on the neurological outcomes of pediatric CM indicates that deficits in this domain typically resolve within six months post-discharge from the hospital (van Hensbroek et al., 1997). Neurocognitive deficits, on the other hand, appear to be more persistent (e.g., Carter et al., 2005b; John et al., 2008). The available literature indicates that neurocognitive deficits frequently occur in the domains of working memory and attention (Boivin et al., 2007). Research has also suggested that pediatric survivors of CM and children diagnosed with ADHD have similar neurocognitive profiles, such that 11% of pediatric survivors in one study also exhibited symptoms of ADHD (Birbeck et al., 2010).

Also, as both CM and traumatic brain injury (TBI) are forms of acquired brain injury, an examination of the pediatric TBI literature may be an appropriate means of elucidating the neurocognitive outcomes one may expect to find following pediatric CM. While CM is the result of a disease process and TBI is the result of an injury, little is known about acquired brain damage in Africa and the closest approximation is the TBI literature. Deficits in attention and executive functioning have also been documented to frequently occur in pediatric TBI cases (e.g., Yeates, et al., 2005). In one investigation, Yeates and colleagues (2005) determined that 20% of children classified with a severe TBI (i.e., GCS $\leq$ 8 and brain abnormalities or sustained loss of consciousness) manifested symptoms of inattention and impulsivity-hyperactivity in accordance with a diagnosis of ADHD-Combined Type. Similarities between children with a

TBI and ADHD have also been demonstrated in investigations of brain structure, particularly with regard to abnormalities in white matter, including the CC. While the results from the current study were not statistically significant, a moderate effect size for CC area was found, indicating that children diagnosed as retinopathy-positive may have smaller overall CC. Thus, the results of this study indicate that the abnormalities in brain structure noted in pediatric survivors of CM are likely consistent with the ADHD and TBI literature.

Recent research also suggests that these neurocognitive deficits may not be present immediately following discharge from the hospital, but instead appear over time. A prospective study of pediatric survivors of CM discovered that a variety of neurological and neurocognitive deficits arose approximately three – ten months following recovery (Birbeck et al., 2010). Specifically, motor, sensory, and language deficits were noted approximately three months following discharge from the hospital. Disruptive behavioral disorders (i.e., ADHD) were then evident in some of the survivors five months later. Lastly, seizures were noted approximately 10 months post-discharge from the hospital. Thus, many deficits, not only neurological, but neurocognitive, may not be present immediately following recovery.

This finding has several clinical implications. First, most children are not followed after discharge from the hospital; however, this article in conjunction with the current study suggests that pediatric survivors of CM should be followed over time, as deficits in functioning may not be evident immediately. Additionally, in light of this finding, it is suggested that at follow-up visits, if children demonstrate poorer progress, additional testing and a new scan should be performed. However, completing follow-up scans regardless of the child's progress would be a beneficial means of elucidating the possible mechanisms by which CM may cause structural changes in the brain. Completing scans at the acute phase of the illness may not allow time for

changes in brain structure to develop. Furthermore, as recent research suggests that many neurocognitive deficits (i.e., disruptive behaviors) are not evident until approximately five months post-discharge from the hospital, follow-up scans at this time period may further elucidate possible connections between anatomical changes in the brain and neurocognitive functioning (Birbeck et al., 2010). Further thorough examinations would allow for a more comprehensive understanding of the child's level of functioning, which would permit interventions to be tailored to the child accordingly.

Future Directions

As the current study is among the first to concurrently investigate retinopathy-positive and negative cases of pediatric CM, future research should continue to investigate whether structural differences exist in the brain of children diagnosed with retinopathy-positive and negative CM. Further research would benefit from a more comprehensive understanding of whether or not these two groups differ in terms of structural changes in the brain. Future research should examine whether grey or white matter is affected, particularly in the retinopathy-positive children to determine why the brains of these children are smaller than the retinopathy-negative children. Also, it is critical to examine the relationship between attention measures and brain structure, particularly concerning the CC. This examination would allow for a more direct assessment of possible associations between neurocognitive functioning and structural findings in the brain. Finally, it is important to begin linking neurocognitive and behavioral data with structural changes in the brain to more fully understand the effect of acquired brain damage on later functioning.

Table 1

Description of Coma Scales

Glasgow Coma Scale		**Blantyre Coma Scale**	
Behavior	Points	Behavior	Points
Eye Opening		Eye Movement	
Spontaneous	4	Watches or follows	1
In response to speech	3	Fails to watch or follow	0
In response to pinprick (pain)	2		
No response	1		
Motor (M)		Best Motor Response	
Follows commands	6	Localizes painful stimulus	2
Can localize pain	5	Withdraws limb from painful stimulus	1
Withdraws from painful stimulus	4	No response or inappropriate response	0
Abnormal flexion to pain	3		
Extensor response to pain	2		
No response	1		
Verbal (V)		Best Verbal Response	
Oriented	5	Cries appropriately with painful stimulus	
Confused conversation	4	or if verbal speaks	2
Inappropriate words	3	Moan or abnormal cry with painful	
Incomprehensible sounds	2	stimulus	1
No response	1	No vocal response to painful stimulus	0

Note. Glasgow Coma Scale source is Semrud-Clikeman & Teeter Ellison (2009). The Blantyre Coma Scale source is Waller et al. (1995); Molyneux, Taylor, Wirima, & Borgsteinj, (1989).

Table 2

Participant Demographics

	Retinopathy-positive		Retinopathy-negative		
	M	*SD*	*M*	*SD*	*p*
Age	47.00	20.98	55.80	40.68	0.04
Coma Score	1.50	0.52	1.50	0.58	1.00
Coma Duration	53.60	21.82	20.50	9.15	0.26
Seizures	0.75	0.50	0.75	0.44	1.00
Parasitemia	195854.40	295848.60	216439.50	360252.70	0.69

Note. Age in months, Coma Score = admitting Blantyre Coma Scale score, Coma Duration in hours, Seizures = history of seizures, Parasitemia = parasitemia count on admission per ul.

Table 3

Correlations for the Measured Variables

	Genu	Body	Midbody	Isthmus	Splenium	CCA	SA	Age
Genu	-							
Body	.76**	-						
Midbody	.76**	.93**	-					
Isthmus	.63**	.75**	.81**	-				
Splenium	.70**	.78**	.74**	.60**	-			
CC Area	-.84**	-.88**	-.87**	-.72**	-.82**	-		
SA	.25	.27	.31	.28	.29	-.02	-	
Age	.26	.26	.27	.23	.23	-.22	.39	-

Note. $^{**}p < 0.001$, CC = corpus callosum, CCA = corpus callosum area, SA = supratentorial area.

Table 4

Means, Standard Deviations, and Effect Sizes for the Retinopathy-Positive and Negative Groups

	Retinopathy-Positive		Retinopathy-Negative		
Measure	*M*	*SD*	*M*	*SD*	*d*
Supratentorial Area	9096.13	527.36	9699.50	557.25	1.11
CC Area	580.06	99.95	642.75	98.89	0.63
5 CC Segments					
Genu	183.51	32.62	198.95	35.98	0.45
Body	90.63	22.88	102.30	18.53	0.56
Midbody	75.41	15.24	84.04	16.11	0.55
Isthmus	73.67	15.77	79.26	17.01	0.34
Splenium	155.35	27.23	176.44	19.55	0.89

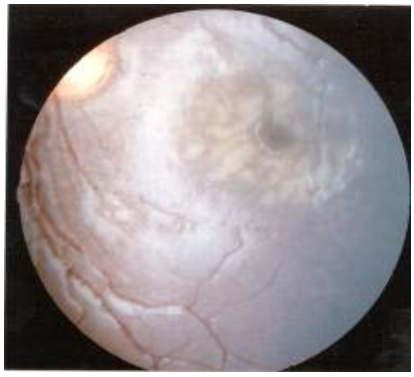
Note. CC = corpus callosum.

Table 5

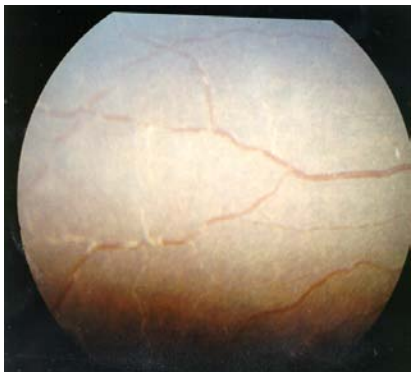
Means, Standard Deviations, and Effect Sizes for Retinopathy-Positive Group

	Males		Females		
Measure	M	SD	M	SD	*d*
Supratentorial Area	9320.00	523.79	8808.29	397.00	1.40
CC Area	566.78	53.18	597.14	143.53	.28

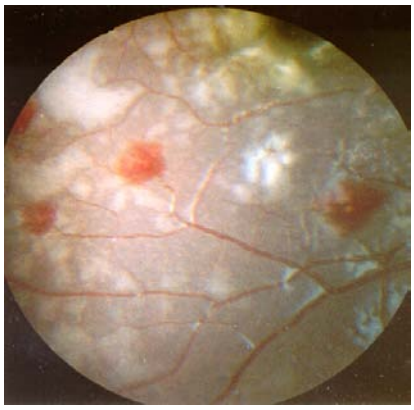
Note. CC = corpus callosum.



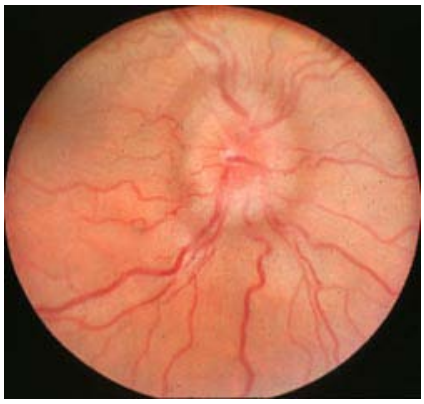
A) Retinal Whitening



B) Vessel Changes



C) Retinal Hemorrhages



D) Papilledema

Figure 1. Retinal findings. For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this thesis. This figure illustrates the four retinal findings documented in pediatric patients diagnosed with cerebral malaria. The pictures are courtesy of Terrie Taylor, DO, Malaria Research Project Ward, Blantyre, Malawi.

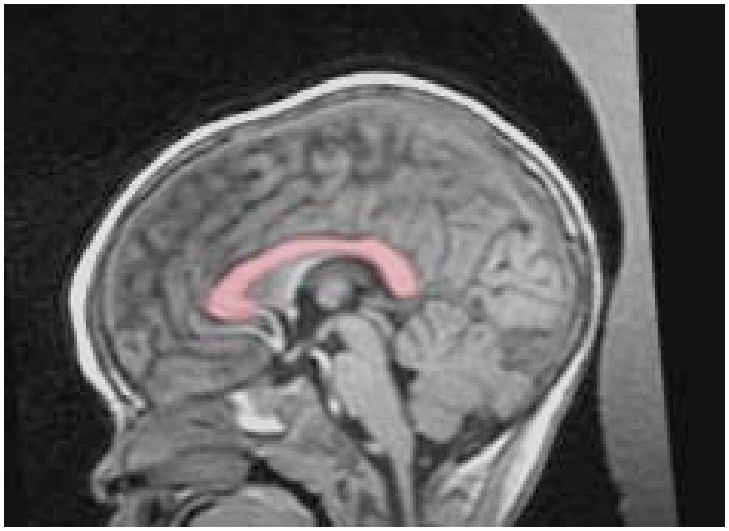


Figure 2. Corpus callosum area measurement. This figure illustrates the corpus callosum area measurement completed for participant.

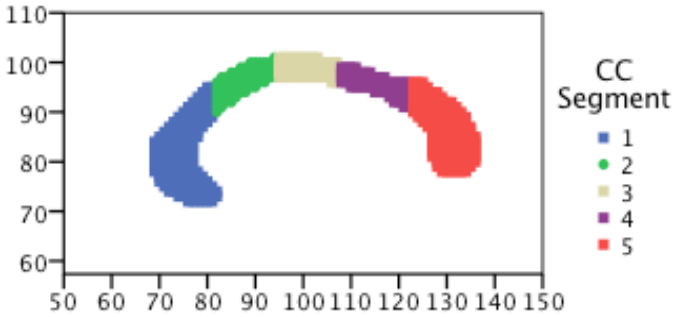


Figure 3. Segmentation of the corpus callosum. This figure illustrates a graphical representation of the corpus callosum following the automatic segmentation process.

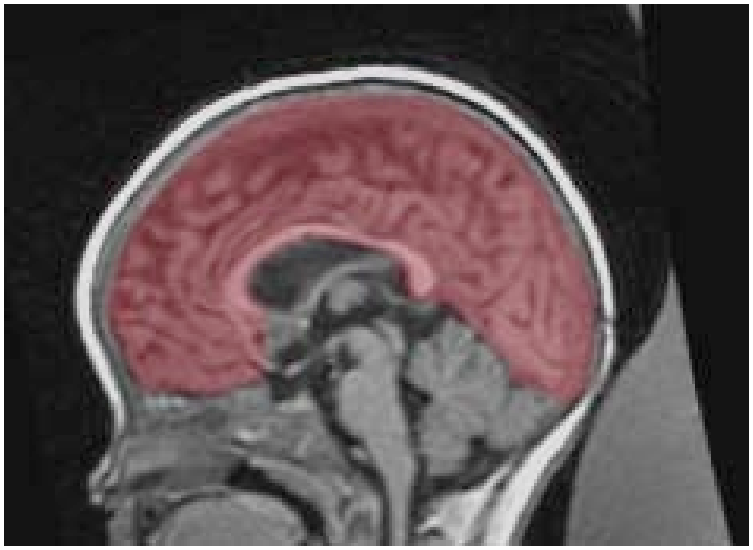


Figure 4. Supratentorial area measurement. This figure illustrates the supratentorial area measurement completed for each participant.

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