Characterizing the Relationship of Histidine-Rich Protein-2 to the Neurologic Sequelae of Severe Malaria

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ABSTRACT

Despite improvements in malaria control and diagnostics, severe malaria continues to claim 800,000 lives every year. *Plasmodium falciparum*, the parasite that most often causes the severe forms of malaria, detoxifies its internal environment in the red blood cell stage of infection through a process mediated by the parasite-secreted histidine-rich protein 2 (pHRP2). pHRP-2 is widely used in diagnostics, yet its role in severe malaria is not well defined.

Therefore, this project aimed to characterize the relationship of pHRP2 to the neurologic sequelae of severe malaria. My hypothesis was that pHRP2 levels would be significantly higher in children with severe disease, as compared to healthy controls and that higher levels would be associated with neurologic sequelae.

pHRP2 levels were measured and compared between plasma samples from children diagnosed with cerebral malaria (CM), severe malarial anemia (SMA) and community controls (CC). CM samples were found to have the highest levels of pHRP2, followed by SMA and then CC. pHRP2 levels were also significantly increased in patients that had neurologic sequestration, as determined by retinopathy, and in patients who died from infection. The strong association between pHRP2 and severe disease, mortality and neurologic sequestration suggests that pHRP2 plays a role in the pathogenesis of severe malaria.

BACKGROUND

Malaria is a complex and life-threating disease that claims approximately 800,000 lives every year. Five different *Plasmodium* species cause malaria in humans, the most dangerous being *Plasmodium falciparum*. *P. falciparum* infections can lead to severe disease defined by presence of the parasite and either coma in cerebral malaria (CM) or severely low hemoglobin levels in severe malarial anemia (SMA) [1]. The burdens of severe malaria are not only the loss of life, but also neurologic deficits, as well as recurrent severe anemia [2] [3]. While numerous studies have investigated severe malarial pathogenesis, the mechanism remains undefined, though it is hypothesized to involve infected erythrocyte sequestration in post-capillary venules of the brain, cytokine mediated inflammation, and hemostasis dysfunction [4].

Plasmodium parasites invade and replicate in host red blood cells, metabolizing red cell hemoglobin as its major energy source for growth and replication [4]. In metabolizing hemoglobin, the toxic byproduct heme is created. To survive, *Plasmodium* detoxifies heme into hemozoin, a process that is mediated in part by the parasite-secreted histidine-rich protein-2 (pHRP-2) [5].Thus, there is a direct correlation between pHRP-2 concentration and number of parasites in the body, including parasites both in peripheral blood and those sequestered in tissues. Subsequently, pHRP-2 is widely used as a biomarker in commercially available malaria rapid diagnostic tests as it is a more accurate measure of parasite biomass as compared to blood smears [5]. Furthermore, sequestration of erythrocytes is associated with more severe disease and therefore high levels of pHRP2 may indicate a greater amount of sequestration.

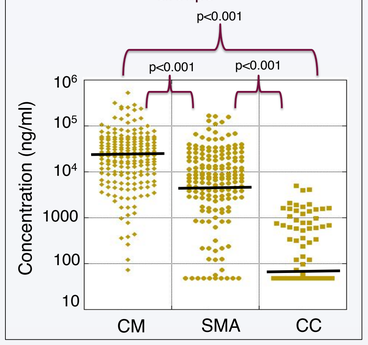
METHODS

The study was conducted at the Mulago Hospital in Kampala, Uganda as a part of a collaborative study assessing complications in cerebral malaria. Children less than 12 years of age were recruited and were assigned to one of three groups: cerebral malaria (CM) (n=197), severe malarial anemia (SMA) (n=170) and community controls (CC) (n=152). CM children were enrolled if met the following World Health Organization criteria: *Plasmodium falciparum* smear positive and the presence of coma with no other cause of encephalopathy. SMA was defined by being *Plasmodium falciparum* smear positive and by having serum hemoglobin concentration ≤ 5 mg. The clinical staff at the Mulago Hospital conducted a series of neuropsychological tests on CM patients in order to accurately assess the effects of the disease on cognitive ability.

pHRP2 levels were quantified from patient plasma samples using the commercially available Malaria Ag CELISA kit (Cellabs, Sydney, Australia). Samples that fell below the detectable range were given a concentration of the lowest detectable value. 10% of samples from each plate were retested on subsequent plates in order to determine consistency between plates. All statistical analysis was completed using STATA version 12.0 (StataCorp, College Station, Texas). Differences were considered significant at $p < 0.05$. Ethical approval for the study was granted by the Institutional Review Board for Human Studies at the University of Minnesota and at Makerere University.

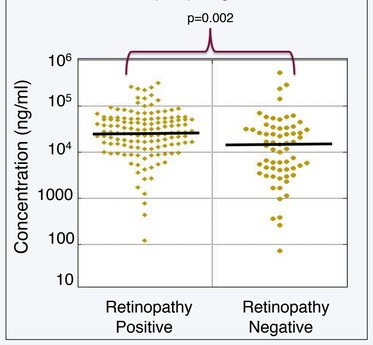
RESULTS

pHRP2 Concentrations Between Disease Groups



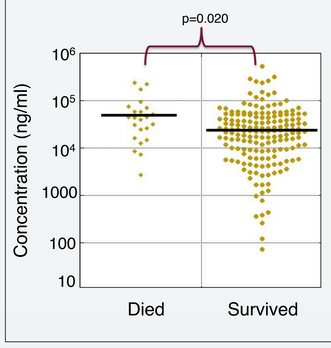
Group	Median	IQR
CM (n=197)	24864 ng/ml	41760 ng/ml
SMA (n=168)	7590 ng/ml	18528 ng/ml
CC (n=152)	48 ng/ml	60 ng/ml

pHRP2 Concentrations Between Retinopathy Positive and Retinopathy Negative CM Patients



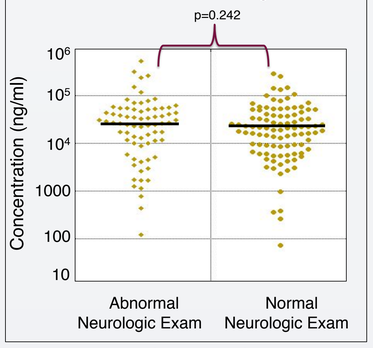
Retinopathy	Median	IQR
Positive (n=133)	27960 ng/ml	38760 ng/ml
Negative (n=62)	14184 ng/ml	28752 ng/ml

pHRP2 Concentrations Between CM Patients Who Died and Survived



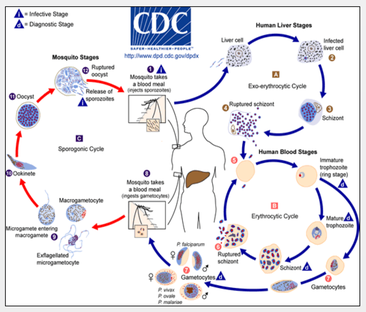
Outcome	Median	IQR
Died (n=23)	44136 ng/ml	55488 ng/ml
Survived (n=173)	22872 ng/ml	40128 ng/ml

pHRP2 Concentrations Between CM Patient Abnormal and Normal Neurologic Exams



Neurologic Exam	Median	IQR
Abnormal (n=73)	26784 ng/ml	41448 ng/ml
Normal (n=99)	20616 ng/ml	33648 ng/ml

- CM samples had the highest median pHRP2 concentration (24,864 ng/ml) followed by SMA (7590 ng/ml) and then community controls (48 ng/ml). pHRP2 levels between all three groups (CM, SMA, and CC) were found to be statistically significant ($p<0.001$).
- A significant difference was also found in pHRP2 levels between those who survived and died from cerebral malaria with increased pHRP2 levels seen in fatal cases ($p=0.020$).
- pHRP2 levels were significantly higher in patients who were retinopathy positive compared to those who were retinopathy negative ($p=.002$).
- pHRP2 concentrations were also compared to the presence of neurologic deficits and the duration of coma, but no statistical significance was found.



The life cycle of a *Plasmodium* parasite. pHRP2 is released during the human blood stage and helps detoxify the environment to ensure parasite survival.

CONCLUSIONS

- Increased pHRP2 levels are not only associated with cerebral malaria, but also severity of disease. SMA, a severe form a malaria with reduced mortality, saw a significant increase in pHRP2 levels as compared to community controls.
- The gold standard for diagnosing cerebral malaria is the presence of retinopathy, or retinal damage, as it is an indicator red blood cell sequestration in the brain. Patients with retinopathy positive cerebral malaria had the highest pHRP2 concentrations. This suggests that patients with high levels of pHRP2 also have erythrocyte sequestration. While the role of pHRP2 in sequestration is unknown, it is an area of research to investigate in the future.
- While retinopathy positive patients did have the highest pHRP2 concentrations, CM patients without retinopathy still had extremely elevated levels (14,184 ng/ml) as compared to SMA patients (7590 ng/ml). Thus retinopathy alone may not be the most accurate definition of CM, as those without retinopathy are still burdened with significantly increased pHRP2.
- pHRP2 levels were significantly higher in CM patients who died, further suggesting the association between the protein and the severity of disease. Fatal cases had the highest median pHRP2 concentration (44,136 ng/ml) suggesting it may have a role in the pathogenesis of severe disease.
- Future direction includes looking at specific cognitive outcomes as compared to pHRP2 levels. We also plan to compare levels to other biomarkers in hopes of understanding the mechanistic role of pHRP2 in pathogenesis.

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