Human Total Tau and its Role as a Possible Biomarker for Cognitive Deficits in Cerebral Malaria

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Abstract

Cerebral malaria (CM) caused by Plasmodium falciparum infection is a major cause of death in children of developing countries, yet the costs of CM are not just death alone. Recent studies have indicated that children who survive the disease have an increased risk of persistent neurological deficits and cognitive impairments. In this study we investigated the cerebral spinal fluid (CSF) levels of the protein tau as a potential predicative measure of the severity of these deficits. Tau is associated with the stabilization and assembly of neuronal microfilaments and is found in the CSF after neuron damage. Elevated levels of tau have been found in children with CM. We hypothesized that levels of tau in CM children would be higher in those children who had neurologic deficits and higher in those who had long-term cognitive impairments, compared to those who did not. We measured tau in the CSF of 142 CM Ugandan children and compared levels to the children's cognitive and neurologic test scores in areas such as working memory, executive attention and learning. Working memory was negatively correlated to tau levels at both time of enrollment and at six months later (P=0.01 rho=-0.3; P=0.02 rho=-0.3). Executive attention and learning were not correlated to tau levels (all P>0.05), and there was no significant difference between those children who had neurologic deficits and those who did not (P=0.5). Investigations of tau levels with cognitive impairments are ongoing, as cognitive testing is still being performed on some children.

Introduction

Cerebral malaria is a severe complication of malarial disease characterized by coma, death and acute cognitive impairments and neurologic deficits. Though it affects approximately 785,000 children every year and kills nearly 20% of those hospitalized (1), the pathogenesis of the disease is still largely unknown (2). We recently conducted prospective study to evaluate the long-term cognitive and neurological effects on children with CM (3). The first aim was to assess areas, frequency and severity of cognitive impairments by testing working memory (using Kaufman assessment battery for children [K-ABC]), attention (using the visual form of the computerized tests of variables of attention [TOVA]) and learning (using the tactile performance test [TPT]). The second aim was to assess presence of neurologic deficits by giving neural exams testing gait, coordination, vision, hearing, movements, sensation, and speech. We found that children with CM had a 3.7-fold increased risk of cognitive impairments compared to healthy children. Risk of deficits was increased if multiple seizures occurred. Likewise, high rates of neurologic deficits were seen at the time of hospital discharge.

Here we investigated the protein tau as a possible biomarker of these long-term cognitive impairments and neuronal deficits. Tau is a protein located in neuronal axons that promotes axon microtubule assembly and stability. Total tau is comprised of phosphorylated and un-phosphorylated tau. It is thought that with axonal damage, the protein is released into the extracellular space and thus the cerebral spinal fluid (CSF). Elevated levels of tau have been seen in several neurological diseases such as Alzheimer's, Creutzfeldt-Jakob, and acute stroke (4,5,6,7), and disease of more intense damage had greater increases in tau levels. Two studies done by Medana et al have also indicated that tau may be a marker of neuronal damage in malaria (8,9), as tau levels were significantly elevated in CM patients compared to controls (either malaria with prostration or malaria with seizures but normal consciousness). Elevated levels of tau were also associated with duration of coma, frequency of convulsions, and anemia.

We hypothesized that levels of tau in CM children would be higher in those children who had neurologic deficits and higher in those who had long-term cognitive impairments, compared to those who did not. If levels of tau correlated to the presence or severity of cognitive impairments and neurologic deficits, tau could be used to identify high-risk individuals and preventative treatment paths could be implemented.

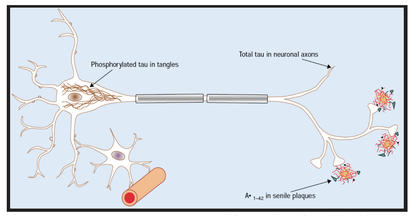
Methods

CSF samples were previously obtained at Mulago Hospital in Kampala, Uganda. The samples were from 142 children ages 5-12 who were recruited as a part of the study assessing complications of CM. Written informed consent was obtained from the parents or guardians of study participants. Ethical approval for the study was granted by the Institutional Review Boards for Human Studies at Makerere University Faculty of Medicine, University Hospitals of Cleveland, Case Western Reserve University, University of Minnesota, and Indiana Wesleyan University.

Tau levels were measured from CSF using a Luminex-based Human Total Tau Antibody Bead Kit (Invitrogen). Luminex is a solid phase multiplex protein immunoassay that uses spectrally encoded antibody conjugated beads as the solid support. Analyte (tau) is bound along with detector antibody and then labeled with a fluorescent conjugate (R-Phycoerythrin, RPE). Levels of tau are analyzed with a Luminex instrument that monitors the spectral properties of the captured beads while simultaneously measuring fluorescence. Fluorescence is then converted to concentration through standard curve generated with the assay.

Testing was initially done on only 74 of the CM samples. These 74 were part of the initial study assessing cognitive impairments mentioned above. The samples were later re-tested, along with 68 new samples from children who were more recently recruited as a part of a continuation study. Controls also tested included children 8-15 years old with inherited metabolic disorders that were treated at the University of Minnesota and children 2-10 years old who had leukemia (in remission).

It was determined that values of total tau were not normally distributed. Wilcoxan rank-sum test was performed to determine if differences existed between tau levels obtained and grouped positive and negative neurologic tests results. Spearman's correlation was used to assess any correlations between total tau levels and ranged representative test scores from the cognitive impairment tests. The representative scores included sequential processing for working memory of the K-ABC test, D-prime test for executive attention of the TOVA, and total time per block for learning of the TPT. An "impaired cognition" summary variable was also compared to tau levels.



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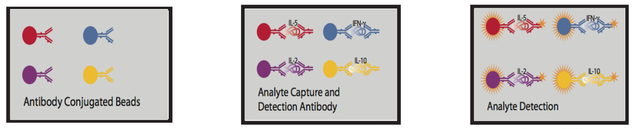
Luminex Assay Overview



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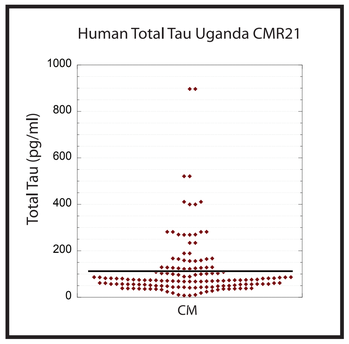
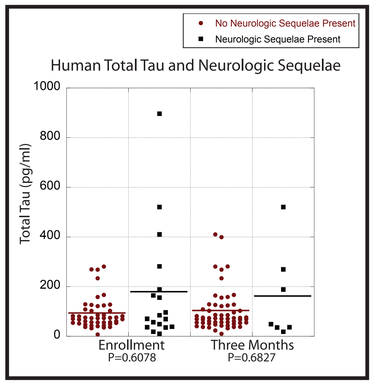


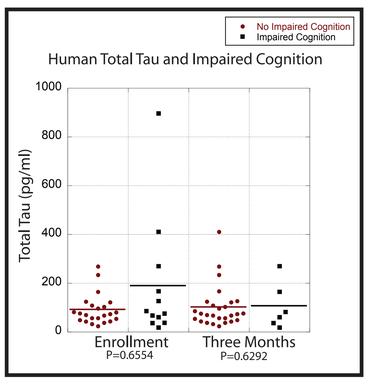
Cognitive Impairment Correlation

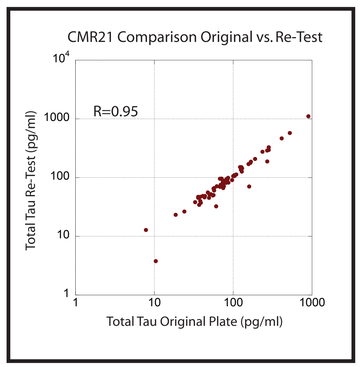
Cognitive Area	Assessment At Enrollment		Assessment at 6 Months	
Attention (n=53)	p=0.1677	rho=-0.2117	p=0.7545	rho=-0.0440
Working Memory (n=59)	**p=0.0189**	rho=-0.2906	**p=0.0295**	rho=-0.2835
Tactile Learning (n=31)	p=0.9639	rho=-0.0085	p=0.9639	rho=-0.0085

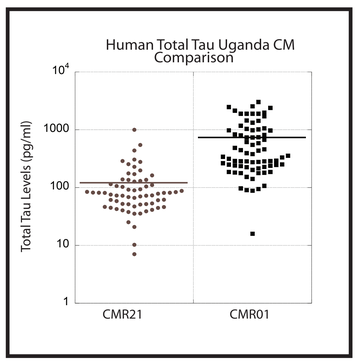
Neurologic Deficit Differences

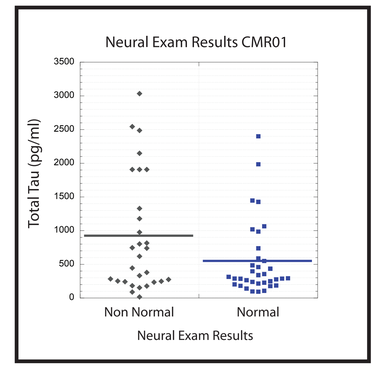
Deficit	At Enrollment	At Three Months
Neurologic Sequela 1=Yes 0=No	**p=0.5404** (n=18, n=46)	**p=0.6971** (n=7, n=55)
Cognitive Impairment 1=Yes 0=No	**p=0.6654** (n=12, n=22)	**p=0.6294** (n=6, n=26)











CMR01 Neurologic Differences

Neural Exam Results	P Value
0=Normal, 1=Non Normal (n=35, n=29)	0.1471

Summary

- Working memory was negatively correlated to tau levels at both time of enrollment and at six months later (P=0.01 rho=-0.3; P=0.02 rho=-0.3).
- Executive attention and learning were not correlated to tau levels (all P>0.05)
- There was no significant difference between those children who had neurologic deficits and those who did not (P=0.5).
- Tau may be affected by freeze thaws.

Conclusions

Levels of total tau were not significantly different in children who had neurologic deficits compared to those who did not. However, working memory appeared to be negatively correlated to total tau levels, indicating tau may be correlated to cognitive impairments.

Future Study

- Compare tau levels to cognitive test variables from new Uganda Study (CMR01) as they are performed
- Compare tau levels to immediate manifestations of CM such as anemia, number of seizures, coma duration, etc.

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Acknowledgements

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