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SHORT REPORT: ROLE OF VIRUSES IN KENYAN CHILDREN PRESENTING WITH ACUTE ENCEPHALOPATHY IN A MALARIA-ENDEMIC AREA

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Abstract. In malaria-endemic areas, it is difficult to differentiate between cerebral malaria (CM), bacterial meningitis, and viral encephalitis. We examined the cerebrospinal fluid of 49 children who fulfilled the World Health Organization's (WHO) definition of CM and in 47 encephalopathic children, without malaria, looking for viruses with polymerase chain reaction. In the children with CM, four (9%) had evidence of Herpes simplex virus 1 in the cerebrospinal fluid, whereas in the encephalopathy group without malaria, six (12%) were positive. A significant proportion of children who fulfil the WHO clinical definition of CM may have viral encephalitis.

In malaria-endemic areas, the diagnosis of falciparum malaria is one of exclusion, because up to 70% of the children in the community may have parasitemia and yet be asymptomatic.[1] Thus, if a patient presents with a febrile illness, this can only be attributed to malaria after exclusion of other causes. This is particularly important in children presenting with impaired level of consciousness. Bacterial meningitis cannot be excluded by clinical examination, but can be excluded by the examination and culture of the cerebrospinal fluid (CSF).[2] However, in resource-poor countries, exclusion of viral encephalitis is more problematic. Little information is available on prevalence and manifestation of the neurotropic viruses in Africa. The contribution of viral pathogens to the encephalopathy syndrome usually attributed to *Plasmodium falciparum* has not yet been assessed. We looked for evidence of herpesviruses and enteroviruses in the children who fulfilled the World Health Organization's (WHO) criteria for cerebral malaria.[3]

Polymerase chain reaction (PCR) was performed to detect DNA of herpes viruses and RNA of enteroviruses in CSF. The microbiological examination and measurement of glucose and protein were as described previously.[2] The residual CSF was stored at –20°C within an hour of sampling and later at –80°C until PCR analysis was performed. DNA of the samples was isolated according to the Boom-method.[4] Positive controls (viral DNA) and negative controls (calf-thymus DNA) were also included. Primers for the enteroviruses and the herpes viruses were selected according to well-established techniques.[4–6] The primer pair sequences and amplification conditions are available on request. The purified PCR products were used for hybridization and were measured by electrochemoiluminescence using the M8 system (M-Series; IGEN, Oxfordshire, U.K.) with streptavidin coated magnetic beads (Dynabeads; Dynal, Oslo, Norway).

We studied 96 children admitted to Kilifi District Hospital (KDH) between 1999 and 2001. KDH is situated in the rural coast of Kenya, in a malaria endemic area, where malaria is the most important cause of childhood morbidity and most frequent cause of admission.

In patients who were unconscious (unable to localize a painful stimulus), a lumbar puncture was performed to exclude central nervous system infections. Examination of the ocular fundus was carried out on admission. Patients with proven or probable bacterial meningitis after blood culture, glucose measurement, or antigen detection were excluded from the study.[2]

We looked for viruses in two clinically defined groups:

1) Cerebral malaria (CM): children who fulfilled the WHO definition[3] (i.e., a child who is unable to localize a painful stimulus and has a peripheral asexual parasitemia and in whom bacterial meningitis and hypoglycemia were excluded as causes of the encephalopathy).
2) Encephalopathy without evidence of malaria: children who were unable to localize a painful stimulus, in whom asexual parasites were not detected in three blood slides taken over 24 hours.

All children were given intravenous antibiotics (benzylpenicillin and chloramphenicol) until the results of the CSF culture were reported, and intravenous quinine if they had asexual *P. falciparum* parasites on their peripheral smear or until three blood slides were negative. Anti-viral medication (e.g. acyclovir) was not available. The Kenya National Ethics committee approved this study.

The clinical details on the children are shown in Table 1. The PCR procedure was successful for all subjects. Herpes simplex virus type 1 (HSV-1) DNA was detected in the CSF of four (9%) children with a clinical diagnosis of CM (group A). No other viral DNA was identified in this group. In children with slide-negative encephalopathy (group B), eight (17%) were positive for viral DNA, six (12%) for HSV-1, one for cytomegalovirus, and one for varicella zoster virus and enteroviral DNA. There was no statistical difference in any of the clinical features between the patients with HSV who had a positive slide ($N = 4$) compared with those who had a negative slide ($N = 6$).

In comparison with the children who fulfilled the definition of CM after exclusion of the viral infections, the children with HSV-1 encephalitis had a significantly higher hemoglobin and longer duration of hospitalization (Table 2). There were no significant differences in age, incidence of convulsions or status epilepticus, axillary temperature, weight, retinal findings on direct ophthalmoscopy, or laboratory results.

Varicella zoster viral DNA and enteroviral RNA were de-

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TABLE 1
Clinical characteristics of children studied

Clinical characteristics	Group A (cerebral malaria)	Group B (slide negative encephalopathy)
Number of children	49	47
Age (months): median (IQR)	22.3 (7.7, 34.9)	2.8 (0.3, 21.9)
Male (%)	30 (61%)	32 (68%)
History		
Duration of history (days): median (IQR)	3.0 (1.5, 3.0)	3.0 (1.0, 3.3)
History of convulsions (%)	41 (84%)	23 (49%)
Status epilepticus: seizure > 30 minutes (%)	11 (22%)	6 (13%)
Examination on admission		
Axillary temperature (° C): mean (SD)	38.1 (1.5)	37.4 (1.2)
Weight (kg): mean (SD)	9.6 (3.7)	6.8 (5.5)
Blantyre coma score ≤ 2 (%)	49 (100%)	47 (100%)
Spleen size (cm): median (IQR)	0.0 (0.0, 2.5)	0.0 (0.0, 0.0)
Laboratory investigations		
White blood cell count ($\times 10^9$/L): median (IQR)	12.0 (9.1, 20.1)	15.5 (9.1, 18.7)
Hemoglobin (g/dL): mean (SD)	6.9 (2.8)	12.1 (3.8)
Platelet count ($\times 10^9$/L): mean (SD)	307 (126)	448 (88)
Parasitemia ($\times 10^6$/L): mean (SD)	28,095 (27,757)	0.0 (0)
Children without parasites (%)	0 (0%)	47 (100%)
Glucose (mmol/L): mean (SD)	5.0 (2.7)	4.5 (2.5)
Children with hypoglycemia (%)	6 (2%)	5 (11%)
Cerebrospinal fluid		
Time between admission and lumbar puncture (hours): median	16.5	1.4
White cell count (/mm^3): median (IQR)	0.0 (0.1, 4)	2.0 (0.1, 16.0)
Protein (g/dL): median (IQR)	0.2 (0.1, 0.3)	0.4 (0.2, 0.7)
CSF/blood glucose ratio: mean (SD)	0.8 (0.3)	0.7 (0.2)
During admission		
Convulsions during admission (%)	13 (27%)	9 (19%)
Outcome		
Duration of hospitalization (days): median (IQR)	3.0 (2.0, 5.0)	6.0 (2.0, 10.0)
Neurological deficits (%)	1 (2%)	2 (4%)
Death (%)	3 (6%)	3 (6%)

IQR, interquartile range (25th and 75th percentile).

tected in a 1-month-old girl who presented with a history of cough before becoming unconscious and unable to localize a painful stimulus. There was no history of convulsions. She had evidence of lower respiratory tract infection. She recovered without any sequelae. Cytomegalovirus was detected in a 6-month-old boy who presented with a 7-day history of fever and cough. On admission to the hospital he was conscious and breast feeding, but had severe wasting and developed a convulsion and focal neurologic deficits. The boy was discharged without any neurologic deficits detectable.

We found that 9% of children who fulfilled the WHO criteria for the diagnosis of CM had evidence of HSV-1 within their CSF. There were no useful clinical features that distinguished between these conditions. In particular, there was no cut-off for age, platelet count, or CSF white cell count that could be used to distinguish between these two conditions.

In malaria-endemic areas, the diagnosis of falciparum malaria is one of exclusion, because many children in the community may have parasitemia and yet be asymptomatic.[1] A recent post-mortem study found that 23% of the children who fulfilled the WHO definition of CM died of other causes.[7] Although virology was not reported in this study, none of the children had pathologic features of encephalitis. Only features of malaria retinopathy were associated with sequestration of parasites in the brain. We were only able to examine the fundi with direct ophthalmoscopy; more thorough examination with indirect ophthalmoscopy may detect differences between HSV-1 encephalitis and CM.

We found that HSV-1 was the most common cause of viral encephalitis in this study. Although we did not look for flaviviruses in this study, a previous study of the CSF from 25 encephalopathic children admitted to KDH did not detect any RNA of 68 known flaviviruses, including West Nile and dengue viruses (L. Dunster, personal communication).

This study suggests that a significant proportion of children who fulfill the WHO definition of CM may have other causes, including viral encephalitis, and this may confound studies on pathophysiology and neurocognitive sequelae. If these results are confirmed by further studies, the research on CM should be reinterpreted with these constraints.

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TABLE 2
Characteristics HSV encephalitis and cerebral malaria

Clinical presentation	HSV-1 encephalitis	Cerebral malaria	*P*
Number of children	10	45	
Age (months): median (IQR)	10.5 (5.4, 30.9)	22.7 (8.7, 36.9)	0.25*
Male (%)	50%	36%	0.48†
History			
Duration of history (days): median (IQR)	5.5 (2.8, 10.0)	3.0 (1.0, 3.0)	0.60*
History of convulsions (%)	8 (80%)	36 (80%)	1.00†
Number of convulsions 24 hours before admission: median (IQR)	3.5 (2.0, 4.0)	1.0 (1.0, 3.0)	0.46*
Status epilepticus: Seizure lasting > 30 minutes (%)	2 (20%)	11 (24%)	0.71†
Examination			
Axillary temperature (° C): mean (SD)	37.5 (1.5)	38.1 (1.5)	0.24‡
Weight (kg): mean (SD)	7.5 (3.7)	9.8 (3.8)	0.08‡
Blantyre coma score ≤ 2 (%)	10 (100%)	45 (100%)	1.00†
Retinal hemorrhages	0/8 (0%)	4/39 (10%)	1.00†
Spleen size (cm): median (IQR)	2.0 (0.0, 3.8)	0.0 (0.0, 2.0)	0.18*
Laboratory features			
White cell count ($\times 10^9$/L): median (IQR)	15.4 (11.8, 27.5)	11.9 (9.1, 20.1)	0.50*
Hemoglobin (g/dL): mean (SD)	9.7 (5.8)	7.0 (2.8)	0.03‡
Platelet count ($\times 10^9$/L): mean (SD)	310 (127)	378 (148)	0.447‡
Children with malaria parasitemia (%)	4 (40%)	45 (100%)	< 0.01†
Sodium (mmol/L): mean (SD)	134.0 (7.2)	134.4 (5.0)	0.80‡
Creatinine (μmol/L): median (IQR)	57.0 (51.3, 75.3)	58.0 (41.5, 74.5)	0.69‡
Blood glucose (mmol/L): mean (SD)	4.2 (1.6)	5.0 (2.8)	0.34‡
Children with hypoglycemia (blood glucose < 2.2 mol/L) (%)	1 (10%)	2 (4%)	0.46†
Cerebrospinal fluid			
White cell count (/mm^3): median (IQR)	0 (0.1, 6.0)	0 (0.0, 4.0)	0.95*
Protein (g/dl): median (IQR)	0.2 (0.1, 0.5)	0.2 (0.1, 0.3)	0.33*
CSF/blood glucose ratio: mean (SD)	0.9 (0.2)	0.8 (0.2)	0.48‡
During admission			
Duration of fever longer than 7 days (%)	1 (10%)	0 (0%)	0.18†
Convulsions during admission (%)	4 (40%)	11 (24%)	0.67†
Outcome			
Duration of hospitalization (days): median (IQR)	5.5 (2.8, 10.0)	3.0 (2.0, 4.5)	0.05*
Neurological deficits (%)	1 (10%)	0 (0%)	0.17†
Death (%)	1 (10%)	3 (7%)	0.56†

* Mann-Whitney test.
† Fisher exact test.
‡ Independent samples *t* test, equal variances assumed.
IQR, interquartile range (25th and 75th percentile).

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