



Complement Utilization in Children with Severe *P. falciparum* Malaria Anemia



Nancy Nyakoe¹, Ronald Taylor² , Joseph N Makumi ³ and John Waitumbi¹

¹Walter Reed Project, Kenya Medical Research Institute, Kisumu, Kenya

²Department of Biochemistry and Molecular Genetics, University of Virginia School of Medicine, Charlottesville, VA 22908 USA

³ School of Pure and Applied Sciences, Kenyatta University, Nairobi, Kenya



Background



- Complement system, important part of innate and adaptive immunity is activated during malaria
- Presence of malaria antigens in circulation either free or bound to antibodies can activate complement
- Malaria antigens are continuously produced:
 - During schizogony
 - As parasitized RBCs are destroyed
- In a malaria holondemic, there is sustained complement activation:
 - Increased consumption of complement components



Objectives



General objective

- Determine whether children with severe malaria have compromised ability to activate complement

Specific objectives

- To determine the hemolytic complement titers (CH50) in children with severe malarial anemia and in those with uncomplicated malaria
- To determine complement activation products (C4a, C3a and C5a) in plasma collected from the two groups of children
- To determine activity of the three complement pathways
- To determine the prevalence of C4 null alleles in the two groups of children



Study site and Design



Study site: Kisumu District
Hospital, Nyanza, Kenya



Study Design:

- 60 children with severe malaria anemia ($Hb \leq 6g/dl$ and asexual Pf parasitemia)
- Age and sex matched controls with uncomplicated malaria (asexual Pf parasitemia, fever, $Hb > 6g/dl$)
- **Ethical considerations:**
- Consent sought from parents/guardians
- Study was approved by KEMRI scientific and ethical committees



Assay Methods



Complement hemolytic assay (CH50)

- Measures amount of serum required to cause 50% hemolysis of sensitized sheep RBC's

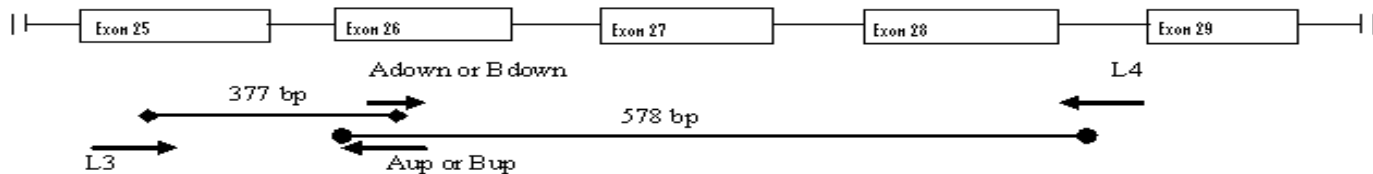
Detection of complement activation fragments

- Levels of C3a-desArg, C4a-desArg and C5a-desArg in plasma measured by ELISA

Determination of functional complement pathways by ELISA

PCR for detection of C4 null alleles (C4AQ0 & C4BQ0)

- Primers specific for C4A and C4B isotypes used for amplification as described by Babra et al, 1994. Eur J Immunogen,21:325-39





Results



Demographics and clinical characteristics of patients

Characteristics	Severe Malaria	Uncomplicated Malaria	P
Sample size (N)	57	44	ND
Age (months)	16.67±9.217	17.02±9.5	ND
Parasitemia (mean ± SD)	97,100±114,000	80700±106,000	0.661
Hb level (g/dl, mean ± SD)	4.5±1.0	8.9±1.3	<0.001

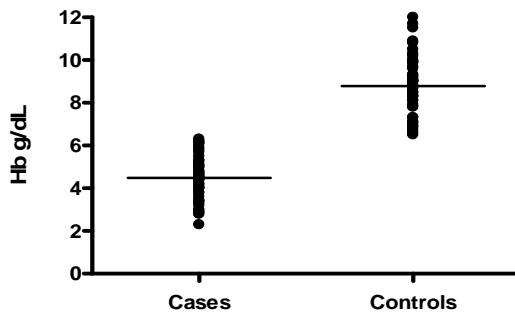


Figure 1. Hemoglobin levels in children with SMA and matched control at enrolment

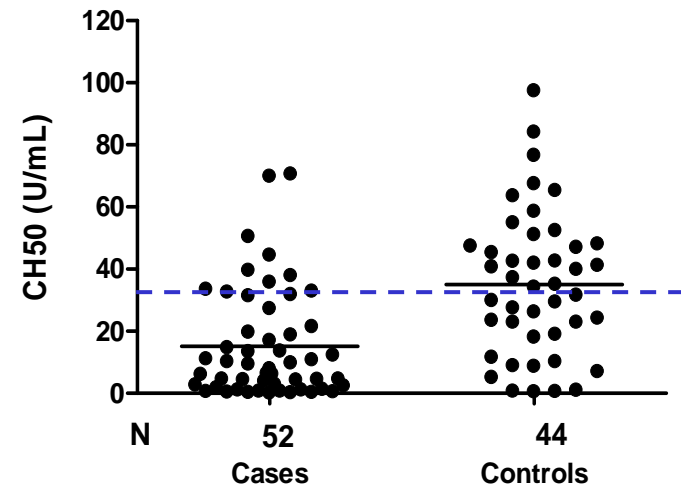
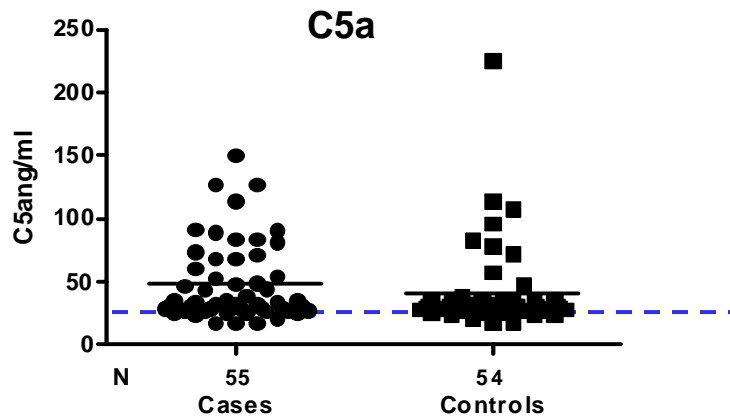
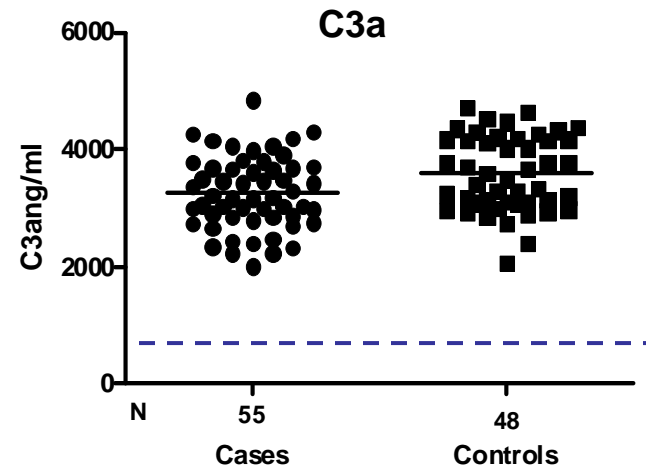
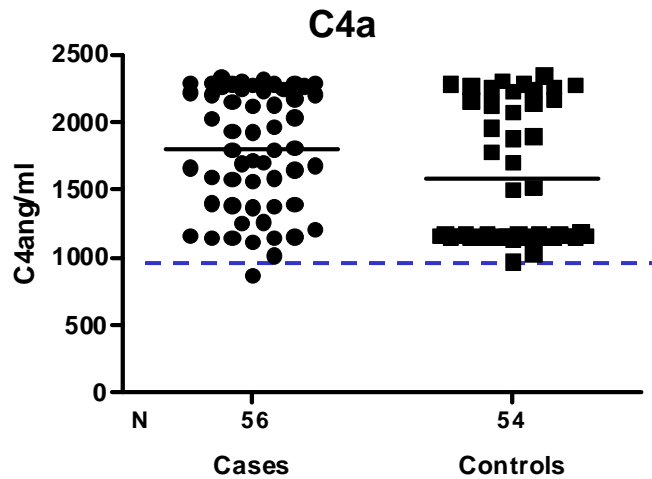


Figure 2: Column plots showing the mean CH50 U/mL in children with SMA (cases) and those with uncomplicated malaria (controls). The mean CH50 (black line) in cases was 15.2 ± 17.6 U/mL compared to 35.0 ± 23.3 U/mL ($P < 0.0001$, paired t test) in children with mild malaria. Paired t test was performed with the 44 paired samples. Dashed line indicate lowest normal levels.

Elevated levels of anaphylatoxins in cases and controls



➤ Figure 3: The mean C4a, C3a and C5a in cases was higher in cases and controls compared to normal levels (dashed line)



Results



All three arms of complement are over-utilized in cases than controls

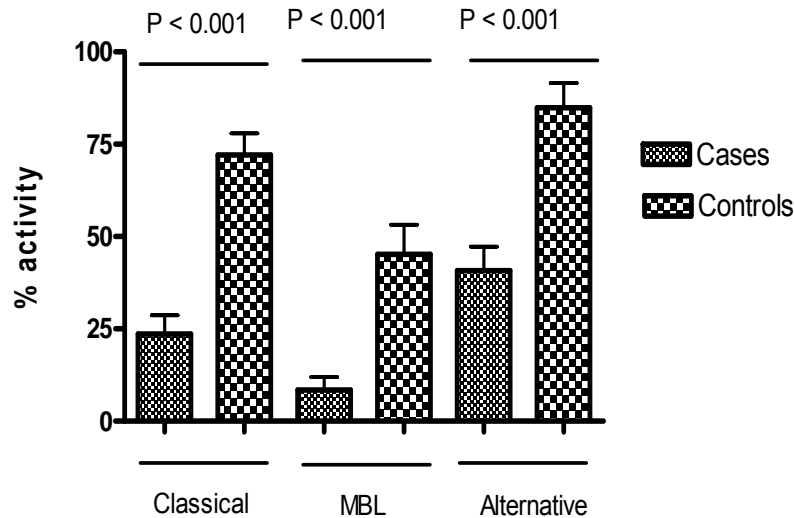
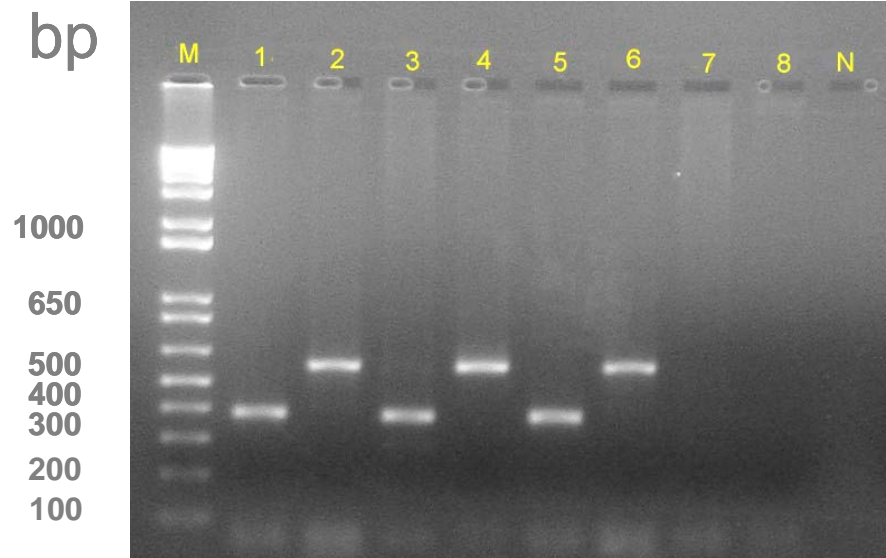


Figure 4: Children with SMA have higher breakdown of complement components than controls



Low complement activity not a result of genetic defects

PCR results for an individual with normal C4 genes i.e. the 378 bp and 578 bp C4A isotypes (lanes 1 and 2 respectively), and the 378 bp and 578 bp C4B isotypes (lanes 3 and 4 respectively).

Lanes 5-8 show PCR products for an individual with normal C4A isotypes (lanes 5 and 6) and null C4B gene (lanes 7 and 8). M is 1 Kb plus molecular weight marker (Invitrogen, CA) and N is a non-template control



Conclusions



- Presence of malaria factors that cause excessive complement utilization irrespective of malarial status
- Excessive utilization of C3 in children with malarial anemia remains uncompensated, thus compromising complement immune defense
- All arms of complement system contribute to the excessive utilization, but CP and MBL are most affected
- Equal formation of pro-inflammatory complement fragments in cases and controls meaning, excess formation of pro-inflammatory cytokines seen in cases of severe malaria anemia cannot be accounted for by these toxins
- Complement deficiency observed in cases of severe malarial anemia are not associated with C4 genetic defects.



Future perspectives



- Complement status of children presenting with malaria should be checked in order to give efficient therapy
- Including antibiotics in the treatment of severe malaria since depletion of complement component leads to inability to protect host against other infections like bacteremia
- Concurrent determination of pro-inflammatory cytokines such as TNF- α and the complement derived pro-inflammatory mediators to define their role of inflammation in severe Malaria



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