Open Forum Infectious Diseases

BRIEF REPORT

Decreased Microvascular Function in Tanzanian Children With Severe and Uncomplicated Falciparum Malaria

Tsin W. Yeo,[1,2,3] **Salvatore M. Florence,**[4] **Ayam R. Kalingonji,**[4] **Youwei Chen,**[5] **Donald L. Granger,**[6] **Nicholas M. Anstey,**[3] **Esther D. Mwaikambo,**[4] **and J. Brice Weinberg**[5]

[1]Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore; [2]Communicable Disease Centre, Institute of Infectious Diseases and Epidemiology, Tan Tock Seng Hospital, Singapore; [3]Menzies School of Health Research and Charles Darwin University, Darwin, Australia; [4]Hubert Kairuki Memorial University, Dar es Salaam, Tanzania; [5]Duke University and V.A. Medical Centers, Durham, North Carolina; and [6]University of Utah and V.A. Medical Centers, Salt Lake City

Microvascular function and oxygen consumption affect oxygen homeostasis but have not been assessed in African children with malaria. Microvascular function in Tanzanian children with severe malaria (SM) or uncomplicated malaria were 39% and 72%, respectively, of controls ($P < .001$). Uncomplicated malaria ($P = .04$), not SM ($P = .06$), children had increased oxygen consumption compared with controls.

Keywords. microvascular function; oxygen consumption; *Plasmodium falciparum*; severe malaria.

A major pathogenic mechanism in severe falciparum malaria is microcirculatory obstruction due to parasite sequestration [1]. However, several studies suggest that sequestration alone may not impair microcirculatory flow in malaria [1–3].

The normal microvasculature matches oxygen delivery and demand, with a major mediator being nitric oxide (NO) [2, 4]. In malaria, NO pathway dysregulation impairs host NO production and bioavailability [5–8]. In Indonesian children, vascular NO and microvascular function was decreased in severe and uncomplicated falciparum malaria [3]. Oxygen demand may exacerbate tissue hypoxia and was increased in Indonesian adults and children with malaria [2, 3]. However, microvascular function and oxygen demand have not been assessed in African children, the group with the highest burden of malaria. We assessed skeletal muscle microvascular function and oxygen consumption in Tanzanian children with severe malaria (SM) or uncomplicated malaria (UM) and compared these to controls.

METHODS

Study Sites and Participants

The study was approved by institutional review boards of the Hubert Kairuki Memorial Hospital, Republic of Tanzania National Medical Research Institute, University of Utah, and Duke University. Informed consent was obtained from parents or guardians of all children.

Children aged 4–12 years old were enrolled if they fulfilled enrollment criteria for SM, UM, or healthy controls (HCs), as previously reported [8]. Younger children were not enrolled because near-infrared resonance spectroscopy (NIRS) (Inspectra 650; Hutchinson Technology, Hutchinson, MN) probes were too large to produce reliable results. Criteria for SM included the following: Plasmodium *falciparum* parasitemia and ≥1 World Health Organization (WHO)-modified criteria for severity, as described previously [9]. Uncomplicated malaria criteria were as follows: a clinical syndrome consistent with malaria and a documented fever (≥38°C) or fever history within 48 hours of enrollment; parasitemia >2500 parasites/μL, positive *P falciparum* rapid diagnostic test ([RDT] Paracheck-Pf; Omega Diagnostics); and no WHO criteria for severe disease. Criteria for HCs included the following: (1) asymptomatic with no febrile illness within the previous 2 weeks and (2) negative *P falciparum* RDT. Exclusion criteria for the overall study were as follows: microscopic evidence of mixed *Plasmodium* infections; bacterial coinfection as evidenced by bacteremia or urinary tract infection; antimalarial therapy initiated >18 hours before enrollment; and hemoglobin <5 mg/dL, because transfusions were not readily available.

Clinical, Laboratory, and Physiological Assessments

History and physical examinations were documented on standardized case record forms. Parasitemia was determined by microscopy, and parasite biomass was determined by *P falciparum* histidine-rich protein 2 using enzyme-linked immunosorbent assay [9]. Hemoglobin, biochemistry, acid-base parameters, and lactate levels were measured with a bedside i-STAT analyzer. The NIRS was performed at enrollment to assess skeletal muscle microvascular function and oxygen consumption, as previously described [2]. In brief, a probe was applied to the thenar eminence, which measured tissue oxygen saturation ([StO_2] expressed as ratio of oxyhemoglobin [O_2Hb]/

Received 27 February 2017; editorial decision 11 April 2017; accepted 19 April 2017.
Correspondence: T. W. Yeo, MD, PhD, Lee Kong Chian School of Medicine, Nanyang Technological University and Imperial College, 11 Mandalay Road, Singapore 308232 (yeotsinwen@ntu.edu.sg).

Open Forum Infectious Diseases®

DOI: 10.1093/ofid/ofx079

Table 1. Baseline Demographics Characteristics, Clinical Features, Hematological, Biochemical, and Microvascular Tests Among Patient Groups[a]

Clinical, Laboratory and Microvascular Parameters	Healthy Control Group (n = 36)	Uncomplicated Malaria (n = 15)	Severe Malaria (n = 48)	P Value[b]
Age, years	8 (6–9)	4 (4–9)	5 (4–9)	$P < .001$
Male sex, no. (%)	22 (61%)	11 (73%)	24 (50%)	$P = .2$
Fever duration before admission (days)	NA	3 (2–6)	3 (2–30)	$P = .5$
Coma, no. (%)	NA	0 (0%)	3 (6%)	$P = .3$
Weight, kg	25 (8–39)	17 (13–23)	16 (11–25)	$P < .001$
Blood Pressure, mm (Hg), Mean (range)				
Systolic	90 (86–105)	90 (80–110)	90 (80–100)	$P = .05$
Diastolic	60 (50–65)	52 (50–60)	57 (50–70)	$P < .001$
Pulse rate, beats/min	87 (78–108)	110 (93–140)	108 (82–160)	$P < .001$
Respiratory rate, breaths/min	24 (20–40)	30 (25–43)	30 (22–56)	$P < .001$
White blood cell count, $\times 10^3$ cells/µL,	7.1 (3.8–11.4)	7.4 (3.2–12.2)	8.9 (2.8–36.8)	$P = .02$
Hemoglobin, g/dL,	12.2 (10.2–13.6)	8.8 (6.1–12,9)	8.5 (4.1–12.9)	$P < .001$
Platelet count, $\times 10^9$ platelets/L,	335 (142–731)	177 (24–720)	80 (8–393)	$P < .001$
Creatinine level, mmol/L,	35.4 (26.5–53.0)	44.2 (26.5–61.8)	35.3 (17.7–70.7)	$P = .19$
Lactate level, mmol/L	1.8 (0.74–2.9)	2.3 (1.4–11.5)	2.8 (1.3–6.6)	$P < .001$
Parasite density, parasite/µL geometric mean (95% CI)	NA	73 306 (48 152–111 600)	237 749 (189 711–297 950)	$P < .001$
HRP2 concentration (ng/mL)	NA	154.9 (1.2–1022)	234.0 (0.8–5896)	$P = .2$
Tissue oxygen saturation, % at baseline	78 (63–93)	82 (75–94)	86 (67–96)	$P < .001$
Tissue hemoglobin index, % at baseline	10.3 (5–14.9)	13.2 (5.5–17.29)	11.8 (4.8–19.6)	$P = .004$
Tissue oxygen saturation, % at end of occlusion	34 (10–56)	39 (18–69)	51 (10–70)	$P < .001$
Tissue hemoglobin index, % end of occlusion	6.7 (2.5–11.5)	7.7 (2.8–12.3)	8.8 (2.3–18.3)	$P = .01$
Peak tissue oxygen saturation after release, %	91 (78–97)	94 (78–98)	93 (71–98)	$P = .6$
Recovery StO_2, % increase/min	249 (57–583)	179 (32–492)	98 (19–480)	$P < .001$
Difference between peak and baseline tissue oxygen saturation, %	11.4 (2–19.4)	5.2 (2.78–12.4)	4.5 (−8.7 to −17)	$P < .001$
Oxygen consumption, arbitrary units	145.5 (51.7–251.5)	167 (94–309.8)	165.5 (70–394.0)	$P = .09$

Abbreviations: CI, confidence interval; HRPT2, histidine-rich protein 2; NA, not applicable; StO_2, tissue oxygen saturation.

[a]All results are median (range), unless otherwise specified.

[b]By Kruskal-Wallis test, comparing the healthy control group, uncomplicated malaria group, and severe malaria group.

sum of oxyhemoglobin [O_2Hb] and deoxyhemoglobin [HHb]) and tissue hemoglobin index ([THI] expressed as sum of relative O_2Hb and HHb signals). Baseline measurements were recorded, after which an ischemic stress was induced by inflating a vascular cuff to 200 mm Hg for 5 minutes, and then rapidly deflating. We recorded the following: (1) baseline StO_2 and THI; (2) StO_2 and THI at the end of occlusion (StO_2low and THIlow); (3) peak StO_2 and THI after release of occlusion (StO_2peak and THIpeak); (4) difference between StO_2 peak and baseline StO_2 (StO_2diff); (5) microvascular function or rate of skeletal muscle reoxygenation (StO_2recov), defined as StO_2 increase per second in the first 14 seconds after occlusion release [12]; and (6) skeletal muscle tissue oxygen consumption (VO2), defined as difference in tissue oxygen content (THI × 1.39 × StO_2) before and after vascular occlusion, divided by the duration [12].

Statistical Methods

Between-group differences among SM, UM, and HCs were compared using an analysis of variance or Kruskal-Wallis test depending on distribution. *A priori* pairwise comparisons using the Sidak method were used to compare CM with UM, as well as CM with HCs, and UM with HCs. A 2-sided *P* value of <.05 was considered to be statistically significant. Pearson/Spearman or partial correlation coefficients were determined as appropriate for the distribution. All analyses were performed on Stata version 12.

RESULTS

We enrolled 99 children (48 with SM, 15 with UM, and 36 HCs) with no deaths recorded. All SM and UM children received anti-malarial therapy according to Tanzanian national protocols (intravenous quinine and artemisinin combination therapy , respectively); 24 SM children also received intravenous antibiotics. Baseline demographic characteristics, clinical features, hematological and biochemical results are summarized in Table 1.

Tissue Oxygen Saturation, Microvascular Reactivity, Oxygen Consumption, and Disease Severity

Physiological measurements were conducted for all children. Baseline StO_2 and THI was higher in SM and UM children compared with HCs (Table 1). The difference between baseline and peak StO_2 values after induction of the ischemic response were significantly lower in SM children compared with UM and HCs (Table 1). Microvascular function at enrollment in the SM and UM groups were 39% and 72% of the median values in HCs, respectively ($P < .001$) (Supplementary Figure 1a). However, there was no significant difference between SM and

UM patients for the difference between peak and baseline StO_2 (P = .2) and microvascular function (P = .3) (Supplementary Figure 1b). There was also no association between microvascular function with peripheral parasitemia or parasite biomass.

Oxygen consumption differed among SMs, UM, and HCs children (P = .06) (Supplementary Figure 1c). On pairwise comparisons, UM children had increased oxygen consumption compared with HCs (P = .04), but the difference between SM patients and HCs was not significant (P = .06). In all children with malaria and those with SM, there was a significant inverse association between oxygen consumption and peripheral parasitemia (r = −0.35 [P = .007] and r = −0.32 [P = .04], respectively) after controlling for disease severity. However, this was not significant in UM patients (r = −0.30; P = .2). There was also no significant association between oxygen consumption and venous lactate or parasite biomass in all malaria patients or the SM and UM groups.

DISCUSSION

Tanzanian children with SM and UM had decreased microvascular function compared with HCs. Uncomplicated malaria but not SM children also had increased skeletal muscle oxygen consumption compared with controls. These findings are the first in African children and consistent with studies in Indonesian Papua, an area with unstable malaria transmission.

In Indonesian adults, we found that microvascular dysfunction was proportional to disease severity, with the most significant impairment in SM [2]. Microvascular function in Indonesian children was lower in SM and UM compared with controls, with no significant difference between the 2 disease groups [3]. In this study, both SM and UM children had median microvascular function values 39% and 72% of HC. However, similar to Indonesian children, microvascular function in SM and UM children were not significantly different. Microvascular function assesses the microcirculatory capacity to match oxygen supply to demand [4]. In microvascular dysfunction, oxygen delivery to normoxic areas are maintained or increased, with flow to hypoxic areas decreased, worsening tissue dysoxia [4]. Parasite sequestration impairs microcirculatory flow, but lack of significant difference between SM and UM children suggests that additional mechanisms may be involved, because parasite biomass is higher in SM [10]. Capillary flow is regulated by precapillary arterioles, with a major mediator being NO [4]. In African children and Indonesian children and adults, systemic and vascular NO bioavailability are markedly reduced in SM and UM [3, 5, 11]. In Indonesian children with malaria, NO bioavailability was associated with microvascular function [3].

Our previous studies have shown increased oxygen consumption in Indonesian adults and children with malaria [2, 3]. A study of Kenyan children with malaria and severe anemia using a metabolic cart found a nonsignificant increase in oxygen consumption, which increased with blood transfusion [12]. In our study, pairwise comparison showed a significant increase in oxygen consumption in UM compared with HCs, but not between SM and HCs. Accentuated oxygen consumption may exacerbate tissue hypoxia by increasing oxygen demand in the setting of impaired delivery. This may explain the higher lactate levels seen in SM, reflecting tissue hypoxia. Microvascular dysfunction could contribute to heterogeneous tissue perfusion observed in falciparum malaria [1], with normal and deceased oxygen delivery to oxygenated and hypoxic regions, respectively. In malaria, decreased NO may increase mitochondrial activity because NO inhibits the electron transport chain [13]. The inverse association between peripheral parasitemia and oxygen consumption in all malaria and SM children suggests that the increased consumption may not be due to parasite metabolism. In contrast, children with bacterial sepsis have decreased oxygen consumption in proportion to disease severity [14]. In malaria, there is an increased macrophage polarization towards an M2 phenotype [9], which is associated with oxidative metabolism compared with bacterial responses, which are polarized to an M1 phenotype associated with aerobic glycolysis [15]. Our present study had several limitations, and the major limitation was the exclusion of children <4 years old due to their inability to use the NIRS probe because of small hand sizes. In addition, the relatively small study size may not have allowed us to detect differences between the SM and UM groups.

CONCLUSIONS

In conclusion, microvascular function is decreased in Tanzanian children with UM and SM, and skeletal muscle oxygen consumption increased in UM. These abnormalities could contribute to impaired oxygen delivery and tissue hypoxia in malaria. Therapies that attenuate or improve microvascular dysfunction may have potential roles as adjunctive therapies in the management of malaria.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Acknowledgments

We thank all patients and their families for participation in our research, and we thank the staff at the Hubert Kairuki Memorial University Hospital in Dar es Salaam, Tanzania. We are indebted to the following study staff for their technical and administrative support: Kokushubila Kairuki, Keto Mshigeni, Rehema Sombi, Stephen Biginagwa, Loyce Kahungya, Kaizerege Karoma, Irene Maokola, Sophia Mbangukura, Stella Shalua and George Kihwili. We also thank the study coordinators (Bernard John, Rebecca Johnson, and Ruth Stanton) for excellent assistance.

Financial support. Financial support for this research was supplied by the US National Institutes of Health (Grants Numbers AI041764 [to J. B. W.] and AI057565 and AI100784 [to D. L. G.]), the US Veterans Affairs Medical Research Service (to J. B. W. and D. L. G.), and the Australian National Health and Medical Research Council (Grant Numbers 1042072 [to N. M. A. and T. W. Y.] and 1037304 [to N. M. A.]).

Potential conflicts of interest. All authors: No reported conflicts of interest.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. White NJ, Pukrittayakamee S, Hien TT, et al. Malaria. Lancet **2014**; 383:723–35.
2. Yeo TW, Lampah DA, Kenangalem E, et al. Impaired skeletal muscle microvascular function and increased skeletal muscle oxygen consumption in severe falciparum malaria. J Infect Dis **2013**; 207:528–36.
3. Yeo TW, Lampah DA, Kenangalem E, et al. Decreased endothelial nitric oxide bioavailability, impaired microvascular function, and increased tissue oxygen consumption in children with falciparum malaria. J Infect Dis **2014**; 210:1627–32.
4. Vallet B. Vascular reactivity and tissue oxygenation. Intensive Care Med **1998**; 24:3–11.
5. Yeo TW, Lampah DA, Gitawati R, et al. Impaired nitric oxide bioavailability and L-arginine reversible endothelial dysfunction in adults with falciparum malaria. J Exp Med **2007**; 204:2693–704.
6. Yeo TW, Lampah DA, Tjitra E, et al. Increased asymmetric dimethylarginine in severe falciparum malaria: association with impaired nitric oxide bioavailability and fatal outcome. PLoS Pathog **2010**; 6:e1000868.
7. Rubach MP, Mukemba J, Florence S, et al. Impaired systemic tetrahydrobiopterin bioavailability and increased oxidized biopterins in pediatric falciparum malaria: association with disease severity. PLoS Pathog **2015**; 11:e1004655.
8. Weinberg JB, Yeo TW, Mukemba JP, et al. Dimethylarginines: endogenous inhibitors of nitric oxide synthesis in children with falciparum malaria. J Infect Dis **2014**; 210:913–22.
9. Weinberg JB, Volkheimer AD, Rubach MP, et al. Monocyte polarization in children with falciparum malaria: relationship to nitric oxide insufficiency and disease severity. Sci Rep **2016**; 6:29151.
10. Rubach MP, Mukemba J, Florence S, et al. Plasma *Plasmodium falciparum* histidine-rich protein-2 concentrations are associated with malaria severity and mortality in Tanzanian children. PLoS One **2012**; 7:e35985.
11. Anstey NM, Weinberg JB, Hassanali MY, et al. Nitric oxide in Tanzanian children with malaria: inverse relationship between malaria severity and nitric oxide production/nitric oxide synthase type 2 expression. J Exp Med **1996**; 184:557–67.
12. English M, Muambi B, Mithwani S, Marsh K. Lactic acidosis and oxygen debt in African children with severe anaemia. QJM **1997**; 90:563–9.
13. Brealey D, Brand M, Hargreaves I, et al. Association between mitochondrial dysfunction and severity and outcome of septic shock. Lancet **2002**; 360: 219–23.
14. Briassoulis G, Venkataraman S, Thompson A. Cytokines and metabolic patterns in pediatric patients with critical illness. Clin Dev Immunol **2001**; 2010:354047.
15. O'Neill LA, Hardie DG. Metabolism of inflammation limited by AMPK and pseudo-starvation. Nature **2013**; 493:346–55.