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

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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) 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 ([StO2] expressed as ratio of oxyhemoglobin [O2Hb]/
sum of oxyhemoglobin [O$_{2}$Hb] and deoxyhemoglobin [HHb]) and tissue hemoglobin index ([THI] expressed as sum of relative O$_{2}$Hb 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$_2$low and THIlow); (3) peak StO$_2$ and THI after release of occlusion (StO$_2$peak and THIpeak); (4) difference between StO$_2$ peak and baseline StO$_2$ (StO$_2$diff); (5) microvascular function or rate of skeletal muscle reoxygenation (StO$_2$ recov), defined as StO$_2$ increase per second in the first 14 seconds after occlusion release [12]; and (6) skeletal muscle tissue oxygen consumption (VO$_2$), 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...
gests that additional mechanisms may be involved, because parasite sequestration impairs microcirculatory flow, but oxygen delivery to normoxic areas are maintained or increased, suggesting delivery to demand [4]. In microvascular dysfunction, oxygen consumption, which increased with blood transfusion [12], 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.

**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].

**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.

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