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MAJOR ARTICLE

Asymmetric Dimethylarginine in Adult Falciparum Malaria: Relationships With Disease Severity, Antimalarial Treatment, Hemolysis, and Inflammation

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Background. Endothelial nitric oxide (NO) bioavailability is impaired in severe falciparum malaria (SM). Asymmetric dimethylarginine (ADMA), an endogenous inhibitor of NO synthase (NOS), contributes to endothelial dysfunction and is associated with mortality in adults with falciparum malaria. However, factors associated with ADMA in malaria, including the NOS-substrate L-arginine, hemolysis, and antimalarial treatment, are not well understood.

Methods. In a prospective observational study of Malaysian adults with SM (N = 22) and non-SM (NSM; N = 124) and healthy controls (HCs), we investigated factors associated with plasma ADMA including the effects of antimalarial treatment.

Results. Compared with HCs, ADMA levels were lower in NSM (0.488 μM vs 0.540 μM, $P = .001$) and in the subset of SM patients enrolled before commencing treatment (0.453 μM [N = 5], $P = .068$), but levels were higher in SM patients enrolled after commencing antimalarial treatment (0.610 μM [N = 17], $P = .026$). In SM and NSM, ADMA levels increased significantly to above-baseline levels by day 3. Baseline ADMA was correlated with arginine and cell-free hemoglobin in SM and NSM and inversely correlated with interleukin-10 in NSM. Arginine and the arginine/ADMA ratio (reflective of arginine bioavailability) were lower in SM and NSM compared with HCs, and the arginine/ADMA ratio was lower in SM compared with NSM.

Conclusions. Pretreatment ADMA concentrations and L-arginine bioavailability are reduced in SM and NSM. Asymmetric dimethylarginine increases to above-baseline levels after commencement of antimalarial treatment. Arginine, hemolysis, and post-treatment inflammation all likely contribute to ADMA regulation, with ADMA likely contributing to the reduced NO bioavailability in SM.

Keywords. ADMA; arginine; malaria; nitric oxide; *Plasmodium falciparum*.

Mortality from severe falciparum malaria (SM) remains high, even with treatment with intravenous artesunate [1]. Development of adjunctive therapies to improve mortality will require improved understanding of the pathogenic mechanisms that underlie severe disease. In falciparum malaria, severe disease results from cytoadherence of parasitized erythrocytes to activated endothelium, leading to microvascular sequestration and obstruction with consequent tissue hypoxia and organ dysfunction [2]. Endothelial nitric oxide (NO) regulates these processes, reducing endothelial activation and downregulating endothelial adhesion molecules [3], inhibiting endothelial cytoadherence of parasitized cells [4], and increasing microvascular flow [5]. Endothelial NO bioavailability is reduced in adults [6] and children [7] with SM and is associated with endothelial activation [8], microvascular dysfunction [7], and impaired tissue perfusion measured by blood lactate [6]. Nitric oxide-dependent endothelial activation and microvascular sequestration of parasitized erythrocytes are both independently associated with microvascular perfusion and mortality in SM [9], suggesting that both processes are important in its pathogenesis.

Production of endothelial NO is largely dependent on the relative concentrations of arginine, the sole substrate for nitric oxide synthase (NOS), and asymmetric dimethylarginine (ADMA), a potent endogenous inhibitor of NOS formed during intracellular methylation of protein-incorporated arginine residues [10]. Asymmetric dimethylarginine is well established as a marker of NO-dependent endothelial dysfunction, and it is elevated in a range of chronic conditions including renal disease, diabetes, hypertension, pulmonary hypertension, and peripheral vascular disease (reviewed in [11]). Asymmetric dimethylarginine is also an independent predictor of cardiovascular events and death in patients with coronary artery disease, renal disease, and diabetes [11], and it is associated with fatal outcomes in patients with sepsis [12].

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Three studies have evaluated the role of ADMA in malaria. In a study of Indonesian adults, almost all of whom had recently started antimalarial treatment, ADMA was increased in SM and associated with mortality [13]. In contrast, enrollment ADMA levels were decreased below the level of healthy controls (HCs) in adults with moderately severe malaria, and they were also decreased in severe and nonsevere malaria in 2 studies of children [14, 15]. However, the effect of prior antimalarial treatment on enrollment ADMA levels has not been investigated.

The relationship between ADMA and arginine in adults with malaria is incompletely understood. Arginine has been shown to regulate concentrations of ADMA through direct inhibition of dimethylarginine-dimethylaminohydrolase (DDAH) [16], the enzyme primarily responsible for the degradation of ADMA, as well as by affecting the activity of the cationic amino acid transporters (CATs) that mediate the transport of both arginine and ADMA between intra- and extracellular compartments [17]. Infusion of arginine increases plasma ADMA [18], and ADMA has been shown to be associated with arginine in adults with critical illness [19, 20] and in children with SM and non-SM (NSM) [15]. The association between ADMA and arginine in adults with malaria has not been assessed.

Given the complexity of arginine and ADMA metabolism in malaria and the importance of ADMA in mediating vascular pathology, we sought to determine the effects of antimalarial treatment on ADMA in Malaysian adults with severe and nonsevere malaria, and we examined relationships with arginine, markers of hemolysis, inflammation, and endothelial NO bioavailability.

METHODS

Study Site and Patients

Patients were enrolled as part of a prospective observational study of all malaria patients admitted to Queen Elizabeth Hospital, an adult tertiary-referral hospital in Sabah, Malaysia [21]. Consecutive patients with polymerase chain reaction (PCR)-confirmed falciparum monoinfection were enrolled from September 2010 to June 2013 (with non-SM patients included until December 2011) if they were nonpregnant, ≥12 years old, had no major comorbidities or concurrent illness, and were within 18 hours of commencing antimalarial treatment. Clinical details of a subset of these patients have been reported previously [21]. Severe malaria was defined as the presence of ≥1 of the following: unrousable coma (Glasgow Coma Scale score <11); multiple (>2) convulsions; respiratory distress (respiratory rate >30 breaths/minute and oxygen saturation <94%); hypotension (systolic blood pressure ≤80 mm Hg); jaundice (bilirubin >43 μmol/L plus parasitemia >100 000 and/or creatinine >132 μmol/L); significant abnormal bleeding; hypoglycemia (blood glucose <2.2 mmol/L); metabolic acidosis (bicarbonate <15 mmol/L or lactate >4 mmol/L); acute kidney injury (creatinine >265 μmol/L); hyperparasitemia (parasitemia >10%). Healthy controls were visitors or relatives of malaria patients, with no history of fever in the past 48 hours and a blood film negative for malaria parasites.

Standardized history and physical examination were documented. Hematology, biochemistry, acid-base parameters, and lactate (by bedside blood analysis; iSTAT system) were obtained on enrollment. Parasite counts were determined by microscopy, and parasite species were identified by PCR [22, 23]. Patients with severe disease were treated with intravenous artesunate, whereas those with nonsevere disease received oral artemisinin combination treatment, as described previously [21].

Laboratory Assays

Venous blood collected in both lithium heparin and citrate tubes was centrifuged within 30 minutes, and plasma was stored at −70°C. Asymmetric dimethylarginine and arginine were measured using reverse-phase, high-performance liquid chromatography with simultaneous fluorescence and ultraviolet-visible detection [24]. Measurements were performed on enrollment and repeated on day 3 in a subset of patients. Plasma concentrations of interleukin (IL)-6 and IL-10 were measured by flow cytometry (BD Cytometric Bead Array). Plasma haptoglobin and cell-free hemoglobin (CFHb) were measured by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (ICL Laboratories and Bethyl Laboratories, respectively). Cell-free hemoglobin was measured from citrated plasma. Parasite biomass was quantified by measuring histidine-rich protein 2 (HRP2) by ELISA [6]. Purified HRP2 was provided by D. Sullivan (John Hopkins University, Baltimore, MD).

Measurement of Endothelial Function

Endothelial function, a measure of endothelial NO-bioavailability, was measured noninvasively using peripheral arterial tonometry (EndoPAT) by the change in digital pulse wave amplitude in response to reactive hyperemia, giving a reactive hyperemia-peripheral arterial tonometry (RH-PAT) index as described previously [6]. The RH-PAT index is at least 50% dependent on endothelial NO production [25] and has been shown to be L-arginine-responsive in falciparum malaria [6]. Endothelial function was measured on enrollment, and it was repeated on day 3 in patients still hospitalized. Measurement of endothelial function was discontinued on patients with nonsevere malaria in May 2011.

Statistical Analysis

Statistical analysis was performed with STATA software (version 10.1). For continuous variables, intergroup differences were compared using analysis of variance or Kruskal–Wallis tests depending on distribution. Student's *t* test or Mann–Whitney *U* tests were used for pairwise comparisons. Categorical variables were compared using χ^2 or Fisher's exact test. Associations between continuous variables were assessed using Spearman's correlation. Multiple linear regression was used to assess for

variables associated with plasma ADMA on enrollment, with variables included in the model if they were significantly associated with ADMA on univariate analysis and were plausibly expected to influence ADMA levels. Multivariable regression was also used to assess for associations with day 3 ADMA, with variables included in the model if they were significantly associated with day 3 ADMA on univariate analysis. The Wilcoxon signed–rank test was used to compare day 0 and day 3 variables.

RESULTS

Patients

A total of 124 patients with NSM and 22 with SM were enrolled. Forty-six (32%) patients were enrolled before commencing antimalarial treatment, including 5 (23%) with SM and 41 (33%) with NSM. Median time to treatment was 3.5 hours and 4.6 hours for patients with SM and NSM, respectively. Of those with SM, 13 patients had 1 severity criterion, 1 had 2 criteria, 7 had 3 criteria, and 1 had 4 criteria. There was no difference in number of severity criteria between patients enrolled before and after commencing antimalarial treatment (median = 1 for both). No patient had coma, and no deaths occurred. Baseline demographics and clinical features are shown in Table 1, and laboratory results in are shown in Table 2.

Baseline Measurements of Asymmetric Dimethylarginine and Effects of Antimalarial Treatment

Plasma ADMA was lower in NSM patients compared with HCs (0.488 μM vs 0.540 μM, $P = .001$) and SM patients (0.569 μM, $P = .009$). There was no difference in ADMA levels between SM patients and HCs ($P = .258$). However, when only patients who had been enrolled before commencing antimalarial treatment were considered, SM patients had ADMA levels as low as those with NSM (0.453 μM [N = 5] vs 0.492 μM [N = 41], $P = .5$) and lower than HCs ($P = .068$). Patients with SM enrolled after treatment had higher ADMA levels than HCs (0.610 μM vs 0.540 μM, $P = .026$). Asymmetric dimethylarginine levels in patients with SM enrolled after treatment were thus significantly higher than in those enrolled before commencing treatment (0.610 μM vs 0.453 μM, $P = .026$). In NSM patients, there was no difference in ADMA levels between those enrolled before or after treatment.

Baseline Measures of Arginine and the Arginine/Asymmetric Dimethylarginine Ratio

Median plasma arginine levels were lower in SM and NSM compared with HCs (64, 62, and 98 μmol/L, respectively, $P = .0001$). Among all patients with falciparum malaria, arginine levels were lower in those enrolled before compared with after commencement of treatment (median 56 μmol/L [N = 46] vs 65 μmol/mL [N = 100], $P = .035$), although this difference was not statistically significant when the severity groups were considered separately (52 μmol/L vs 69 μmol/L [$P = .182$] in SM patients enrolled pre- [N = 5] and post- [N = 17] treatment, respectively, and 56 μmol/mL and 64 μmol/mL [$P = .086$] in NSM patients enrolled pre- [N = 41] and post- [N = 83] treatment, respectively).

The arginine/ADMA ratio (reflective of arginine bioavailability) was lower in SM and NSM patients compared with HCs (106, 127, and 187, respectively, $P = .0001$) and lower in SM compared with NSM ($P = .017$). No difference was seen in the arginine/ADMA ratio between patients enrolled pre- or posttreatment.

Association Between Arginine and Asymmetric Dimethylarginine

Arginine and ADMA were positively correlated in patients with NSM ($r = 0.41$, $P < .0001$), with this correlation being stronger in patients enrolled pretreatment ($r = 0.50$, $P = .0008$, N = 41) compared with those enrolled posttreatment ($r = 0.39$, $P = .003$, N = 83). In SM patients, there was a nonsignificant association between arginine and ADMA ($r = 0.38$, $P = .085$). Asymmetric dimethylarginine and arginine were also weakly associated in HCs ($r = 0.27$, $P = .059$).

Table 1. Baseline Characteristics of Falciparum Malaria Patients and Healthy Controls

Baseline Characteristics	Controls (n = 79[a])	Nonsevere Falciparum Malaria (n = 124)	Severe Falciparum Malaria (n = 22)	*P* Value (Severe vs Nonsignificant)
Age, years				
Median (IQR)	35 (23–44)	27 (18–39)	34 (19–46)	.115
Range	14–69	13–78	13–60	
Males, n (%)	57 (72)	90 (73)	16 (73)	.607
Fever duration, days; median (IQR)		5 (3–7)	7 (5–7)	.093
Systolic blood pressure, mmHg	123 (15)	116 (17)	113 (18)	.411
Pulse rate, beats/min	71 (12)	92 (18)	97 (17)	.239
Respiratory rate, breaths/min	20 (3)	27 (6)	31 (7)	.004
Temperature, °C	36.5 (0.4)	37.9 (1.1)	37.9 (1.3)	.974
Patients enrolled before commencing malaria treatment	NA	41 (33%)	5 (23%)	.336
Time from malaria treatment to enrollment, hours; median (IQR)	NA	4.6 (0–12)	3.5 (0.17–10)	.729

Abbreviations: IQR, interquartile range; NA, not applicable; SD, standard deviation.

[a] Includes all healthy controls/malaria patients who had endothelial function measured and/or blood analysis performed. Numbers are mean (SD) unless otherwise indicated.

Table 2. Laboratory and Physiological Measurements Among Patients With Falciparum Malaria and Healthy Controls[a]

Laboratory/Physiological Measurement	Controls	Nonsevere Falciparum Malaria (n = 124)	Severe Falciparum Malaria (n = 22)	*P* Value (Cont vs Nonsevere vs Severe	*P* Value (Severe vs Nonsevere Pf)
Parasite count (parasites/μL)		10 895 (3866–32 420)	58 301 (9079–273 909)		.0008
HRP2 (ng/mL)		66 (26–216)	541 (125–1590)		<.0001
Hemoglobin, g/dL, mean (SD)		13.0 (1.84)	12.5 (2.62)		.280
Hemoglobin day 3, g/dL, mean (SD)		12.08 (1.81)	10.38 (1.67)		.0002
Hemoglobin fall, g/dL, mean (SD)[b]		1.14 (1.10) N = 86	1.87 (1.81) N = 20		.022
CFHb, μM	15 146 (9641–25 256) N = 50	25 431 (13 371–43 213) N = 110	35 322 (22 023–49 943) N = 21	.0008	.310
CFHb day 3, μM		15 208 (7889–38 539) N = 50	28 563 (15 843–48 559) N = 15		.207
CFHb fall, μM[c]		3676 (−2192–16 420)	4048 (−2933–23 807)		.227
Plasma LDH (μL)	213 (174–290) N = 50	311 (253–404) N = 111	398 (277–578) N = 21	.0001	.035
Haptoglobin[d] (g/L)	1.44 (1.01–1.72) N = 60	1.19 (0.23–1.89) N = 109	0.34 (0.10–2.00) N = 21	.238	.153
AST (U/L)		34 (27–56)	49 (28–81)		.056
IL-6 (pg/mL)	BDL (27 of 30)	46 (19–90)	112 (42–357)	.0001	.0006
IL-10 (pg/mL)	BDL (29 of 30)	180 (85–372)	464 (211–1135)	.0001	.001
L-arg (μmol/L)	98.4 (80.1–112.8) N = 51	62.1 (48.4–83.4)	63.9 (51.2–78.6)	.0001	.956
ADMA (μM)	0.540 (0.490–0.595) N = 51	0.488 (0.428–0.542)	0.569 (0.519–0.651)	.001	.009
L-arg/ADMA ratio	187 (153–213)	127 (98–161)	106 (93–139)	.0001	.017
Endothelial function (RH-PAT index)	1.97 (1.64–2.27) N = 79	1.65 (1.39–1.96) N = 73	1.58 (1.26–1.96) n = 18	.0006	.362
Lactate, mmol/L		1.22 (0.91–1.61) N = 106	1.95 (1.03–2.6) N = 19		.006

Abbreviations: ADMA, asymmetric dimethylarginine; arg, arginine; AST, aspartate transaminase; BDL, below the detection limit; CFHb, cell-free hemoglobin; Cont, controls; HRP2, histidine-rich protein 2; IL, interleukin; IQR, interquartile range; LDH, lactate dehydrogenase; Pf, Plasmodium falciparum; RH-PAT, reactive hyperemia-peripheral arterial tonometry; SD, standard deviation.

[a] Investigations were performed on enrollment, unless otherwise stated. Numbers are median (IQR) unless otherwise stated. Where a result is BDL, half the lower limit of detection is substituted for statistical analyses.

[b] Hemoglobin fall was defined as enrollment hemoglobin minus hemoglobin day 3. The *P* value for the difference in hemoglobin between day 0 and day 3 was .0008 for severe malaria, and <.0001 for nonsevere malaria.

[c] CFHb fall was defined as enrollment CFHb minus day 3 CFHb. The *P* value for the difference in CFHb between day 0 and day 3 was .061 for severe malaria and .007 for nonsevere malaria.

[d] Haptoglobin was BDL in 1 of 60 controls, 11 of 109 patients with nonsevere falciparum malaria, and 3 of 21 patients with severe falciparum malaria.

Association Between Asymmetric Dimethylarginine, Hemolysis, and Inflammation

Asymmetric dimethylarginine was positively correlated with (1) CFHb in patients with SM and (2) plasma lactate dehydrogenase (LDH) in patients with SM and NSM, and it was inversely correlated with haptoglobin, hemoglobin, and IL-10 in patients with NSM (Table 3). There were no significant associations between baseline ADMA and other laboratory variables tested, including HRP2, lactate, aspartate aminotransferase (AST), creatinine, and bilirubin.

In a multivariable model including disease severity, arginine, CFHb, and IL-10 as predictor variables (with continuous variables log transformed), arginine, CFHb, and disease severity remained significantly associated with ADMA ($P < .0001$, $P = .010$, $P = .082$, respectively, $R^2 = 0.2$) (Table 4).

Endothelial Function

As previously reported, endothelial function (as measured by the RH-PAT index) was lower in patients with SM compared with HCs (RH-PAT 1.58 vs 1.97, $P = .003$) (Table 2). However, endothelial function was also impaired in patients with NSM (RH-PAT 1.56), with no difference between the severity groups. No relationship was seen between enrollment endothelial function and ADMA, arginine, or the arginine/ADMA ratio, in either severity group.

Longitudinal Measurements of Asymmetric Dimethylarginine, Arginine, the Arginine/Asymmetric Dimethylarginine Ratio, and Endothelial Function

Asymmetric dimethylarginine levels increased from day 0 to day 3 in patients with SM and NSM (Figure 1), with median concentrations at day 3 in both groups being significantly higher than those of HCs (0.719 μM, 0.700 μM, and 0.540 μM, respectively; $P = .006$ for NSM vs controls, and $P < .0001$ for SM vs controls). Day 3 ADMA was correlated with baseline ADMA in patients with NSM ($r = 0.75$, $P < .0001$, N = 27) but not SM ($r = 0.12$, $P = .819$, N = 7). In patients with NSM, day 3 ADMA was correlated with the fall in hemoglobin from enrollment to

Table 3. Factors Associated With Baseline ADMA in Patients With Severe and Nonsevere Falciparum Malaria[a]

	Nonsevere Falciparum Malaria (N = 124)		Severe Falciparum Malaria (N = 22)	
Variable	R	*P* Value	R	*P* Value
HRP2	0.129	.155	0.144	.533
Arginine	0.411	<.0001	0.346	.085
CFHb	0.183	.055	0.708	.0003
LDH	0.219	.022	0.564	.008
Haptoglobin	−0.234	.014	−0.141	.543
Hemoglobin	−0.223	.013	0.154	.495
AST	−0.008	.932	0.162	.471
IL-6	−0.170	.077	−0.046	.845
IL-10	−0.203	.035	−0.273	.232
RH-PAT	−0.164	.166	0.017	.948
Lactate	0.188	.054	0.068	.781

Abbreviations: ADMA, asymmetric dimethylarginine; AST, aspartate transaminase; CFHb, cell-free hemoglobin; HRP2, histidine-rich protein 2; IL, interleukin; LDH, lactate dehydrogenase; RH-PAT, reactive hyperemia-peripheral arterial tonometry.

[a] R = Spearman's correlation coefficient.

day 3 ($r = 0.43$, $P = .041$) and baseline AST ($r = 0.51$, $P = .007$). In a multivariate model incorporating baseline ADMA, fall in hemoglobin, and AST, all 3 variables remained significantly associated with day 3 ADMA ($P = .004$, $P = .02$, and $P < .0001$, respectively, $r^2 = 0.75$) in patients with NSM. In patients with SM, baseline IL-10 correlated strongly with day 3 ADMA ($r = 0.89$, $P = .007$), although the small numbers (n = 7) did not allow for multivariate analysis.

Arginine also increased from day 0 to day 3 in patients with SM and NSM (median day 3 concentration 107 μmol/mL and 105 μmol/mL, respectively), with the day 3 concentration being significantly higher than HCs (median 98.4 μmol/mL) in patients with NSM ($P = .028$) but not SM ($P = .30$). No correlation was seen between baseline arginine level and day 3 arginine in either severity group. There was also no correlation between day 3 arginine and day 3 ADMA in either severity group, nor any correlation between the increase in ADMA and the increase in arginine. The arginine/ADMA ratio increased from day 0 to day 3 in both severity groups, although it remained below the level of HCs.

Endothelial function improved from day 0 to day 3 in patients with NSM patients (median RH-PAT 1.70 vs 1.92, $P = .001$, N = 38), with a nonsignificant improvement in SM (1.63 vs 1.87, $P = .100$, N = 15). In patients with NSM, but not SM, endothelial function on day 3 correlated with day 3 ADMA ($r = -0.61$, $P = .027$, N = 13) and baseline ADMA ($r = -0.41$, $P = .009$, N = 39). There was no correlation between (1) the increase in RH-PAT and (2) the increase in ADMA, arginine, or the arginine/ADMA ratio.

Table 4. Multivariate Model of Factors Associated With ADMA[a]

Variable	Regression Coefficient	*P* Value
Arginine	0.251	<.0001
Cell-free hemoglobin	0.047	.010
IL-10	−0.026	.099
Severe malaria	0.132	.008

Abbreviations: ADMA, asymmetric dimethylarginine; IL, interleukin.

[a] All continuous variables were log transformed. $R^2 = 0.277$.

DISCUSSION

L-arginine bioavailability, as measured by the arginine/ADMA ratio, was inversely related to disease severity, as previously reported in adult [13] and pediatric severe malaria [14, 15], and likely contributes to the impaired NO bioavailability found in severe malaria. As previously reported, ADMA was reduced acutely in adults with NSM. Although ADMA was higher in SM compared with NSM overall, ADMA levels were lower in the subset of SM patients enrolled before commencing antimalarial treatment, and levels were significantly higher in SM patients enrolled within hours of commencing antimalarial therapy. In addition, ADMA levels increased significantly from day 0 to day 3 in SM and NSM. Taken together, these findings suggest that ADMA is reduced acutely in malaria infection, but it increases rapidly to above-baseline levels after commencement of antimalarial treatment.

Our previous study in Indonesian adults showed that patients with SM had ADMA levels that were markedly higher than those of controls. Although this may relate to the greater severity of disease seen in the Indonesian study, with critical illness and multiorgan failure known to be associated with increased ADMA levels [20], it may also relate to the fact that these patients were enrolled a median of 8 hours after commencement of treatment. This would be supported by the findings of the current study, in which adults with SM enrolled after treatment had ADMA levels significantly higher than those of controls. An acute increase in ADMA after antimalarial treatment in severe malaria is also supported by previous findings in children with falciparum malaria, where ADMA levels were reduced acutely in SM and NSM but increased within 1–2 days to above-baseline levels [14].

A transient reduction in ADMA has been consistently demonstrated in other acute infectious and noninfectious, noncritical, inflammatory conditions. In a study involving patients with sepsis, ADMA levels were elevated in those with severe sepsis but reduced below the level of HCs in those with sepsis without shock [12]. Likewise, in a study involving healthy kidney donors, unilateral nephrectomy was followed by an abrupt decrease in ADMA in association with increasing inflammatory markers [26]. In patients undergoing knee arthroplasty, a procedure known to be associated with significant inflammation, ADMA levels fell by 31%, reaching a nadir on day 2 before recovering to baseline [27]. In a rat model, lipopolysaccharide

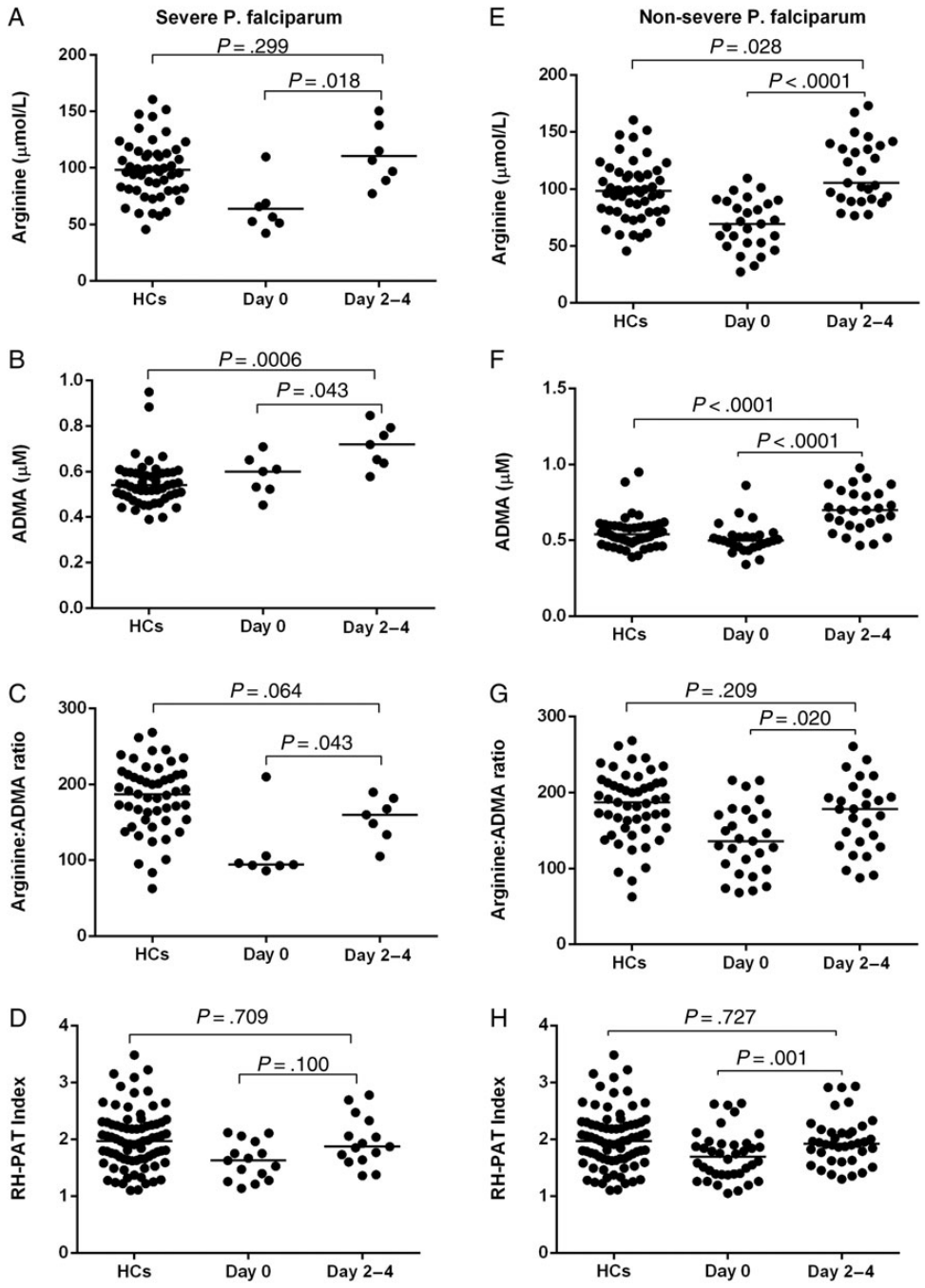

Figure 1. Time course of plasma arginine, plasma asymmetric dimethylarginine (ADMA), the arginine/ADMA ratio, and endothelial function, in severe and nonsevere falciparum malaria, compared with controls. A–D show time course (day 0 compared with day 2–4) of arginine (A), ADMA (B), the arginine/ADMA ratio (C), and endothelial function (D) in patients with severe malaria. E–H show the time course (day 0 compared with day 2–4) of arginine (E), ADMA (F), the arginine/ADMA ratio (G), and endothelial function (H) in patients with nonsevere falciparum malaria. Day 0 values are represented only for the subset of patients who had measurements performed on day 2–4. Median values of baseline arginine, ADMA, arginine/ADMA ratio, and endothelial function among all patients with severe and nonsevere malaria, and comparison of these values with controls, are shown in Table 2. RH-PAT, reactive hyperemia-peripheral arterial tonometry.

(LPS)-induced endotoxemia also resulted in ADMA levels below those of control rats [28].

The explanation for the acute and transient reduction in ADMA during acute inflammation has not been fully determined. Asymmetric dimethylarginine has previously been shown to be inversely associated with C-reactive protein and IL-6 in acute inflammation [19, 29], and in our study, baseline ADMA was inversely correlated with IL-10 in patients with NSM. In rat smooth muscle cells, DDAH is upregulated by IL-1β [30]. Therefore, it has been hypothesized that cytokine-

mediated upregulation of DDAH may contribute to increased clearance of ADMA in acute inflammation [31]. However, in the knee arthroplasty study, urinary dimethylamine, the major metabolic product of ADMA, was measured as an indirect marker of DDAH activity, and excretion was not found to change during the early postoperative period [27]. Furthermore, hepatic DDAH1 activity was shown to be decreased in *Plasmodium berghei*-infected mice, despite reduced concentrations of plasma ADMA [15].

An alternative hypothesis for the decrease in plasma ADMA in acute inflammation is redistribution of ADMA from extra- to intracellular compartments [27]; indeed, in the above-mentioned mouse model in which plasma ADMA concentrations were reduced, hepatic concentrations of ADMA were increased [15]. Circulating ADMA is primarily taken up by the liver and kidneys via CATs, which also transport other cationic amino acids (CAAs) including arginine [32]. Cationic amino acid transporters have been shown to be upregulated by several factors including inflammation [33], and in a rat model expression of CAT-1 was increased in heart, lung, and kidney tissue after LPS injection [34]. In mice, increased expression of CAT-1 was associated with increased cellular uptake of ADMA [35]. Thus, it is possible that upregulation of CATs may lead to increased cellular uptake of ADMA and reduced plasma concentrations. Although upregulation of CATs may also be associated with increased cellular uptake of arginine (and hence may be expected to result in net efflux of ADMA [33]), it is possible that in the setting of plasma arginine deficiency preferential cellular uptake and/or reduced efflux of ADMA occurs. The consequent intracellular accumulation of ADMA could explain the impaired endothelial function seen in SM and NSM in this study, despite low pretreatment plasma ADMA levels [6].

A notable finding in our study was the correlation at baseline between ADMA and arginine. This association has been demonstrated previously in adults with acute inflammation [19, 20] and in children with falciparum malaria [15], and an increase in ADMA levels has been demonstrated after intravenous [18] and oral [36] arginine administration. These findings may relate to the known arginine-mediated inhibition of DDAH [16]. Alternatively, it is possible that competitive transport through the shared CAT accounts for the association between arginine and ADMA. Previous studies have demonstrated (1) CAA-induced release of ADMA into the plasma [36] (2) as well as arginine-induced augmentation of CAT-1 transport activity [37].

In our study, baseline ADMA was also associated with plasma CFHb and LDH in patients with SM and NSM, with the correlation being stronger in SM. Red blood cell (RBC) lysis has been shown to release large amounts of free ADMA [38], and it is likely that hemolysis is an important regulator of ADMA levels in severe malaria. In patients with NSM, day 3 ADMA levels were significantly associated with the fall in hemoglobin, suggesting that treatment-related destruction of RBCs may contribute to the posttreatment elevation of ADMA. Release of ADMA from RBCs may also occur in response to low extracellular concentrations of ADMA, and hence the low ADMA associated with malaria may in itself contribute to the subsequent increase in ADMA concentrations [38].

The early increase in ADMA after commencement of antimalarial treatment in this study is consistent with other studies showing an increase in ADMA with evolution of acute inflammation [27, 29, 31] and with a study of pediatric falciparum malaria [14]. In patients with SM in our study, day 3 ADMA was strongly associated with enrollment (mostly posttreatment) IL-10, and it is possible that the inflammatory response to treatment may contribute to an increase in ADMA. Day 3 ADMA was also associated with baseline AST in patients with NSM, suggesting that hepatic dysfunction may contribute to the posttreatment ADMA accumulation. The liver is known to play a central role in the metabolism of ADMA, being the predominant reservoir of DDAH-1, and ADMA has been shown to correlate with the degree of hepatic dysfunction in patients with cirrhosis, alcoholic hepatitis, and acute liver failure (reviewed in [39]). In mice infected with *P berghei* ANKA, hepatic ADMA clearance was reduced as a result of the inactivation of DDAH-1 [15]. In severe malaria, hepatic ischemia-reperfusion (I/R) injury may also contribute to the increase in ADMA concentrations after commencement of antimalarial treatment. Dimethylarginine-dimethylaminohydrolase-1 activity has been shown to be reduced after hepatic I/R in rats, with serum ADMA increasing in the early reperfusion period [40]. Finally, the increase in ADMA seen during recovery from malaria infection may relate to increasing arginine, due to the aforementioned competitive transport through the CAT-1, or inhibition of DDAH. Importantly, in our study, despite the increase in ADMA that occurred after treatment, endothelial function also improved. In previous larger longitudinal studies in SM, improvement in endothelial function has been associated with recovery of plasma L-arginine concentrations [41], and L-arginine infusion in moderately SM improves endothelial function [6]. Thus, whereas the recovery of endothelial function after treatment may be multifactorial, it is probable that it is in part accounted for by an improvement in NO bioavailability (as indicated by an increase in arginine/ADMA ratio). Nonetheless, in NSM, day 3 ADMA levels were inversely associated with endothelial function, suggesting some residual inhibition of endothelial NO bioavailability, either directly through intracellular inhibition of endothelial NOS or possibly through inhibition of cellular uptake of arginine [33].

Our study had several limitations. In particular, only 5 patients with SM were enrolled before commencing antimalarial treatment, limiting our ability to analyze this subgroup. In addition, ADMA levels were measured only on enrollment and on

day 3, so we were unable to precisely define the time course of ADMA after commencement of antimalarial treatment. Finally, the nature of our study does not allow us to determine the mechanisms of the changes in ADMA concentrations.

CONCLUSIONS

In conclusion, ADMA was reduced acutely (1) in patients with NSM and (2) in patients with SM enrolled before antimalarial treatment; however, in both groups, ADMA levels rose to above-baseline after treatment. Although the factors regulating ADMA in malaria remain uncertain, arginine, hemolysis, and inflammatory cytokines likely play important roles in determining initial ADMA concentrations, whereas further hemolysis, treatment-related inflammation, and hepatic dysfunction may contribute to the posttreatment increase. Given the previously demonstrated association between ADMA and mortality in severe malaria, agents targeting ADMA regulation may have potential as adjunctive treatments of severe malaria.

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