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Suppressed circulating bicyclo-PGE_2 levels and leukocyte COX-2 transcripts in children co-infected with *P. falciparum* malaria and HIV-1 or bacteremia

Samuel B. Anyona [a,b], Prakasha Kempaiah [c], Gregory C. Davenport [c], John M. Vulule [d], James B. Hittner [e], John M. Ong'echa [a], Douglas J. Perkins [a,c,*]

[a] University of New Mexico, Laboratories of Parasitic and Viral Diseases, Centre for Global Health Research, Kenya Medical Research Institute, Kisumu, Kenya
[b] Department of Medical Biochemistry, Maseno University, Maseno, Kenya
[c] Center for Global Health, University of New Mexico, Albuquerque, NM, USA
[d] Centre for Global Health Research, Kenya Medical Research Institute, Kisumu, Kenya
[e] Department of Psychology, College of Charleston, Charleston, SC, USA

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ABSTRACT

In holoendemic *Plasmodium falciparum* transmission regions, malarial anemia is a leading cause of childhood morbidity and mortality. Identifying biomarkers of malaria disease severity is important for identifying at-risk groups and for improved understanding of the molecular pathways that influence clinical outcomes. We have previously shown that decreased cyclooxygenase (COX)-2-derived prostaglandin E_2 (PGE_2) levels are associated with enhanced clinical severity in cerebral malaria, malarial anemia, and malaria during pregnancy. Since children with malaria often have increased incidence of additional infections, such as bacteremia and HIV-1, we extend our previous findings by investigating COX-2 and PGE_2 in children with falciparum malaria and co-infection with either bacteremia or HIV-1. Plasma bicyclo-PGE_2/creatinine levels and peripheral blood COX-2 transcripts were significantly reduced in co-infected children relative to those with malaria mono-infection. Furthermore, suppression of circulating bicyclo-PGE_2 was significantly associated with reduced hemoglobin levels in both mono- and co-infected children with malaria, suggesting that bicyclo-PGE_2 may represent both a marker and mediator of malaria pathogenesis.

1. Introduction

Plasmodium falciparum malaria is the most prevalent form of malaria in sub-Saharan Africa. Severe malaria in African children often presents as a diverse clinical spectrum, ranging from mild infections to life-threatening complications such as severe anemia, cerebral malaria, hypoglycemia, acute renal failure, and acidosis/respiratory distress [1–3]. In falciparum malaria holoendemic transmission regions of western Kenya, severe malarial anemia (SMA; Hb < 6.0 g/dL, with any density parasitemia) is the primary clinical manifestation of severe disease [4], often exacerbated by the presence of co-infections, including HIV-1, bacteremia, and upper respiratory tract viral infections [5–7].

Central to the pathogenesis of malarial anemia is the release of host innate immune mediators generated in response to parasite products [4]. Our laboratory has identified clinical predictors associated with enhanced pathogenesis of SMA [8] and inflammatory biomarkers of pediatric severe anemia [9]. Additional investigations revealed unique hematological predictors and inflammatory mediator patterns associated with the worsening anemia observed in children co-infected with falciparum malaria and HIV-1 [10,11]. Among the various effector molecules implicated in pathogenesis of severe malaria is cyclooxygenase (COX)-2-derived prostaglandin E_2 (PGE_2), a potential biomarker that is inversely associated with disease severity in cerebral malaria, malarial anemia, and malaria during pregnancy [12–14]. In addition, we recently discovered that suppression of COX-2-derived PGE_2 in children with falciparum malaria was associated with reduced erythropoiesis and worsening anemia [15]. Given the important role of co-infections in contributing to severe malaria pathogenesis [5,7], the current study investigated the COX-2-PGE_2 pathway in children with malaria that were co-infected with either bacteremia or HIV-1.

COX-2 (prostaglandin endoperoxide H synthase-2) is an inducible enzyme expressed in cells involved in inflammatory reactions [16,17], and when up-regulated by pro-inflammatory mediators,

* Corresponding author. Address: Center for Global Health, Department of Internal Medicine, MSC10-5550, 1 University of New Mexico, Albuquerque, NM 87131, USA. Fax: +1 505 272 8441.
E-mail address: dperkins@salud.unm.edu (D.J. Perkins).

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can generate high levels of PGE_2 to modulate the host-immune response to infections [18–22]. Since PGE_2 and its metabolites are unstable *in vivo*, levels of PGE_2 are measured as bicyclo-PGE_2 (the stable breakdown product of PGE_2 and 13,14-dihydro-15-keto PGE_2) and can be expressed relative to creatinine levels to account for differences in hydration status [23,24].

2. Materials and methods

2.1. Study site

The study was carried out at the Siaya District Hospital (SDH) in western Kenya. Although the last comprehensive malaria prevalence survey was conducted more than a decade ago and indicated ~83% infection in children between one and four years [25], from mid-2006 to date the area is characterized by an increase in pediatric malaria admissions [26]. In this holoendemic *P. falciparum* transmission region, malarial anemia is the primary cause of hospital-associated morbidity and mortality [27]. Information about the study site and malarial anemia in the pediatric population are described in our previous report [28].

2.2. Study participants

Parasitemic children (aged 3–36 months; $n = 101$) were recruited at SDH after their parents/guardians provided informed written consent. All children were screened for HIV-1 infection using two rapid serological antibody tests and confirmed for HIV-1 positivity by pro-viral DNA according to our previous methods [5]. None of the children were receiving antiretroviral medication at the time of recruitment. Parents and/or guardians received pre- and post HIV-1/AIDS counseling. Bacteremia was determined according to our previous methods [7]. Based on screening results, children were grouped into three categories: those with falciparum malaria alone [*Pf*(+), $n = 74$], malaria plus bacteremia [*Pf*(+)/Bac(+), $n = 19$], or malaria plus HIV-1 [*Pf*(+)/HIV-1(+), $n = 8$]. Children were excluded from the study if they had mixed malaria species infections, prior hospitalization (for any reason), antimalarial and/or antipyretic treatment within two weeks prior to enrollment, and/or cerebral malaria. Patients were treated and provided supportive care according to the Ministry of Health (MOH)-Kenya guidelines. The study was approved by the Ethics Committees of the Kenya Medical Research Institute and University of New Mexico Institutional Review Board.

2.3. Laboratory evaluations

Venipuncture blood samples (<3.0 mL) were collected from enrolled participants before any treatment interventions. Complete blood counts were determined using the Beckman Coulter A^{c}T diff2™ (Beckman-Counter Corporation, Miami, FL, USA). Asexual malaria trophozoites in thick and thin peripheral blood smears, and reticulocyte count were determined according to previous methods [29]. Inflammatory mediator patterns (cytokines and growth factors) were determined in children ($n = 62$) grouped into [*Pf*(+), $n = 40$], [*Pf*(+)/Bac(+), $n = 15$], and [*Pf*(+)/HIV-1(+), $n = 7$] using a Cytokine 25-plex Antibody Bead Kit, Human (BioSource™ International, CA, USA) as previously described [9,10]. Sickle cell trait (HbAS) and glucose-6-phosphate dehydrogenase (G6PD) deficiency were determined according to our published methods [30].

2.4. Determination of bicyclo-PGE_2 and creatinine levels and COX-2 gene expression

Since PGE_2 has a high turnover rate in peripheral circulation, the PGE_2 metabolites (13,14-dihydro-15-keto PGA_2 and 13,14-dihydro-15-keto PGE_2) were converted to single derivatives (stable end product bicyclo-PGE_2). Bicyclo-PGE_2 levels were measured in plasma samples according the manufactures' instructions (Cayman Chemical Company, MI, USA) and our recently reported methods [15].

Plasma creatinine levels were determined using the creatinine determination kit (Cayman Chemicals Company, MI, USA). Plasma samples were diluted 1:20 with ultra-pure water and creatinine was quantified by enzyme immunoassay (ELISA) according to the manufacturers' protocol (Cayman Chemicals Company, MI, USA).

Total RNA was isolated from cryo-preserved white blood cell pellets (preserved in commercial RNAlater® RNA stabilization reagent [Qiagen, CA, USA]) by the acid guanidinium thiocyanate-phenol-chloroform extraction method [31]. Reverse transcription of RNA to complementary DNA (cDNA) was performed using the high capacity cDNA reverse transcription kit (Applied Biosystems, CA, USA) and our previously published reaction conditions [15]. Resulting complementary DNA (cDNA) was amplified for 30 cycles using oligonucleotides spanning the exon–intron junction in the COX-2 gene, with the sense (5′-GAC TCC CTT GGG TGT CAA AGG TAA-3′) and antisense (5′-GTG AAG TGC TGG GCA AAG AAT G-3′) sequence to generate a 138 bp product according to our previous methods [15]. To normalize the amount of cDNA loaded per reaction, an internal control, the cyclophilin A (CYC-A) housekeeping gene was amplified in a 25 μL reaction, containing final concentrations of 0.3 μM each CYC-A sense oligo 5′-GTC TCC TTT GAG CTG TTT GC-3′ and antisense oligo 5′-AAG CAG GAA CCC TTA TAA CC-3′. Resulting fragments were resolved on 2% agarose gel stained with 0.5 mg/mL ethidium bromide (Sigma Chemicals Co. MO, USA) and visualized under UV (Sprectroline® Corporation, NY, USA). Electrophoretic gel films were analyzed using the ImageJ software [32] and PCR product mean band intensities quantified. The COX-2 mRNA expression mean values (arbitrary units; AU) were normalized by expressing them as ratios to CYC-A mRNA mean values.

2.5. Statistical analyses

Analyses were computed using SPSS statistical software package 19 (IBM SPSS Inc., IL, USA). Proportions were compared using Pearson's chi-square analysis, while across-group comparisons performed using Kruskal–Wallis test for median and ANOVA for mean levels. Bivariate analysis was performed using Mann–Whitney *U* test and Student's *t*-tests for bicyclo-PGE_2/creatinine levels and COX-2 mRNA transcripts, respectively. Correlation analysis was performed using Spearman's rank test or Pearson's zero-order rank test. Statistical significance was considered at $P \leqslant 0.050$.

3. Results

3.1. Clinical and laboratory characteristics

Children enrolled in the study ($n = 101$) were stratified into three groups: malaria alone [*Pf*(+); $n = 74$], malaria plus bacteremia [*Pf*(+)/Bac(+); $n = 19$], and malaria plus HIV-1 [*Pf*(+)/HIV-1(+); $n = 8$]. As shown in Table 1, the demographic and clinical characteristics were comparable across groups. Similarly, hematological measures, erythropoietic and parasitological parameters, and genetic variants showed no significant across group differences. Although phagocytosis of *P. falciparum* hemozoin by monocytes is a driving factor for inflammatory mediator production [4], the percentage of pigment-containing monocytes was similar across the groups. Plasma bicyclo-PGE_2 levels were decreased in children with co-infection ($P < 0.001$), whereas plasma creatinine levels

Table 1
Characteristics of study participants.

Characteristics	*Pf*(+)	*Pf*(+)/Bac(+)	*Pf*(+)/HIV-1(+)	*P*
Sample size (*n*)	74	19	8	
Gender, *n* (%)				
Female	29 (39.2)	12 (63.2)	5 (62.5)	0.105[a]
Male	45 (60.8)	7 (36.8)	3 (37.5)	
Age, months*	10.5 (10.0)	10.0 (12.0)	7.0 (13.0)	0.547[b]
Enrollment temperature, °C*	37.7 (1.6)	38.0 (1.9)	38.0 (0.8)	0.540[b]
Severe malarial anemia (Hb < 5.0 g/dL), *n* (%)	22 (29.7)	5 (26.3)	4 (50.0)	0.448[a]
Severe malarial anemia (Hb < 6.0 g/dL), *n* (%)	36 (48.6)	9 (47.4)	5 (62.5)	0.742[a]
Hematological measures				
Hemoglobin, g/dL*	6.2 (3.3)	6.0 (3.1)	5.2 (3.5)	0.847[b]
Hematocrit, %	19.1 (9.8)	19.7 (6.8)	16.8 (12.2)	0.921[b]
Red blood cells, $\times 10^6/\mu L$*	2.6 (1.4)	2.9 (1.8)	2.5 (1.8)	0.737[b]
Red cell distribution width, %*	21.3 (5.1)	23.9 (5.7)	21.7 (11.7)	0.712[b]
Mean corpuscular volume, fL*	70.7 (11.2)	69.2 (10.9)	73.2 (14.8)	0.394[b]
Mean corpuscular hemoglobin, fL/cell*	22.9 (4.0)	22.0 (3.5)	22.3 (5.5)	0.248[b]
Mean corpuscular hemoglobin concentration, g/dL*	32.3 (2.0)	31.8 (3.5)	30.3 (2.8)	0.050[b]
White blood cells, $\times 10^9/L$*	11.1 (8.3)	10.6 (8.5)	10.4 (11.6)	0.932[b]
Lymphocytes, $\times 10^3/\mu L$*	5.7 (4.2)	5.8 (5.0)	5.8 (7.5)	0.850[b]
Monocytes, $\times 10^3/\mu L$*	1.2 (1.1)	0.9 (0.9)	1.2 (0.8)	0.421[b]
Granulocytes, $\times 10^3/\mu L$*	4.6 (4.7)	5.0 (4.4)	4.0 (3.6)	0.933[b]
Platelets, $\times 10^3/\mu L$*	0.12 (0.1)	0.17 (0.2)	0.12 (0.1)	0.088[b]
Mean platelet volume, fL*	8.1 (1.4)	8.1 (1.4)	9.3 (1.9)	0.260[b]
Platelet distribution width, %	17.6 (1.3)	18.0 (1.7)	17.6 (1.1)	0.752[b]
Erythropoietic parameters				
Reticulocyte production index*	0.83 (1.2)	0.71 (1.4)	0.91 (1.7)	0.691[b]
RPI < 2, *n* (%)	66 (89.2)	18 (94.7)	6 (75.0)	0.356[a]
Parasitological parameters				
Parasite density, MPS/μL*	10,439 (33,027)	6514 (25,525)	22,174 (37,660)	0.345[b]
Geometric mean parasitemia, /μL	9564	5227	15,301	0.687[c]
High density parasitemia ($\geqslant$10,000/μL), *n* (%)	38 (51.4)	7 (36.8)	6 (75.0)	0.186[a]
Genetic variants				
Sickle cell trait, *n* (%)	10 (13.5)	5 (26.3)	0 (0.0)	0.176[a]
Glucose-6-phosphate dehydrogenase deficiency, *n* (%)	5 (6.9)	1 (5.9)	1 (20.0)	0.542[a]
Other laboratory parameters				
*Pf*Hz containing monocytes, *n* (%)	36 (48.6)	8 (42.1)	3 (37.5)	0.761[a]
Plasma bicyclo-PGE_2, pg/mL*	11.3 (8.8)	4.9 (2.8)	4.9 (4.4)	<0.001[b]
Plasma creatinine, mg/mL*	0.45 (0.56)	0.62 (0.23)	0.75 (0.29)	0.004[b]

* Data presented as median (interquartile range, IQR). Children were stratified into those with malaria [*Pf*(+)] alone, malaria plus bacteremia [*Pf*(+)/Bac(+)], and malaria plus HIV-1 [*Pf*(+)/HIV-1(+)], all with any density parasitemia.
[a] Pearson's $\chi 2$ test was used to determine differences in proportions.
[b] Kruskal–Wallis test was used to compare differences across groups.
[c] Analysis of variance (ANOVA) was used to compare geometric mean parasitemia. MPS – Malaria parasites.

were elevated during co-infection ($P = 0.004$), relative to those with malaria mono-infection.

3.2. Bicyclo-PGE_2 and COX-2 transcript levels

To determine the effect of co-infection on PGE_2 production, we assayed the stable end-metabolite of PGE_2 in circulation (bicyclo-PGE_2, pg/mL) and normalized across the groups by expressing the levels relative to creatinine (bicyclo-PGE_2/creatinine, pg/mg/mL). Analyses of bicyclo-PGE_2/creatinine levels revealed that the *Pf*(+)/Bac(+) group had significantly decreased plasma levels compared to the *Pf*(+) group ($P < 0.001$; Fig. 1A). In addition, the *Pf*(+)/Bac(+) group had significantly lower ($P = 0.033$) peripheral blood COX-2 transcripts relative to the *Pf*(+) group (Fig. 1B). Analysis of PGE_2 production in the *Pf*(+)/HIV-1(+) group revealed that co-infection was associated with decreased plasma bicyclo-PGE_2/creatinine concentrations compared to children with malaria mono-infection ($P < 0.001$, Fig. 1C). In addition, the *Pf*(+)/HIV-1(+) co-infected group had lower COX-2 mRNA transcript levels relative to children with malaria mono-infection ($P < 0.001$; Fig. 1D).

3.3. Relationship between bicyclo-PGE_2 and anemia

To determine if there was a relationship between PGE_2 production and anemia, the association between circulating bicyclo-PGE_2/creatinine levels and Hb were determined for the overall cohort, and for each of the groups separately. Spearman's rank correlation analyses revealed a significant positive relationship between bicyclo-PGE_2/creatinine and Hb concentrations in the overall cohort ($r = 0.330$, $P = 0.001$; Fig. 2A), *Pf*(+) group ($r = 0.363$, $P = 0.002$; Fig. 2B), *Pf*(+)/Bac(+) group ($r = 0.595$, $P = 0.007$; Fig. 2C), and *Pf*(+)/HIV-1(+) group ($r = 0.819$, $P = 0.013$; Fig. 2D). These results illustrate that circulating bicyclo-PGE_2/creatinine levels are a valid biomarker for disease severity in children with malaria as a single infection and in the context of co-infection with either bacteremia or HIV-1.

3.4. Relationship between bicyclo-PGE_2 and inflammatory mediator production

During a malaria infection, there are counter-regulatory effects between COX-2-derived PGE_2 and a number of cytokines including, tumor necrosis factor (TNF)-α, interferon (IFN)-γ, and interleukin (IL)-10 [4]. Investigation of this established immunological network revealed that plasma TNF-α levels were elevated in the *Pf*(+)/HIV-1(+) group ($P = 0.126$), while IFN- γ was highest in *Pf*(+)/Bac(+) children ($P = 0.056$), and that IL-10 was comparable across the groups ($P = 0.393$) (Table 2). Further exploration using Pearson's zero order correlation test demonstrated no significant relationships between circulating bicyclo-PGE_2 levels and TNF-α

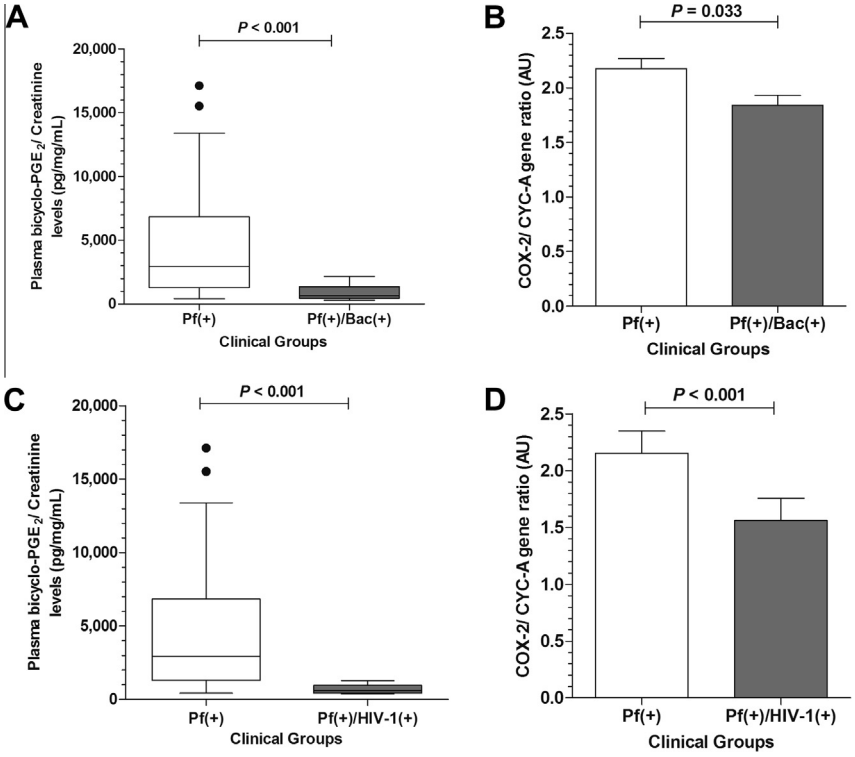

Fig. 1. Systemic bicyclo-PGE_2 and COX-2 mRNA expression in children with malaria and co-infected with either bacteremia or HIV-1. (A) Plasma bicyclo-PGE_2/creatinine ratio in children with malaria alone [*Pf*(+)] versus malaria and bacteremia [*Pf*(+)/Bac(+)]. (B) Semi-quantitative COX-2 gene expression expressed relative to CYC-A in arbitrary units (AU) in children with malaria alone [*Pf*(+)] versus those co-infected with bacteremia [*Pf*(+)/Bac(+)]. COX-2 mRNA expression is presented as means, with the error bars showing the standard error of mean (SEM). (C) Plasma bicyclo-PGE_2/creatinine ratio among children with malaria alone [*Pf*(+)] versus those with malaria plus HIV-1 [*Pf*(+)/HIV-1(+)]. (D) Semi-quantitative COX-2 gene expression expressed relative to CYC-A (AU) in children with malaria alone [*Pf*(+)] versus those with malaria plus HIV-1 [*Pf*(+)/HIV-1(+)]. Plasma levels were compared using Mann–Whitney *U* test. Differences in COX-2 gene expression between groups were computed using Student's *t*-test.

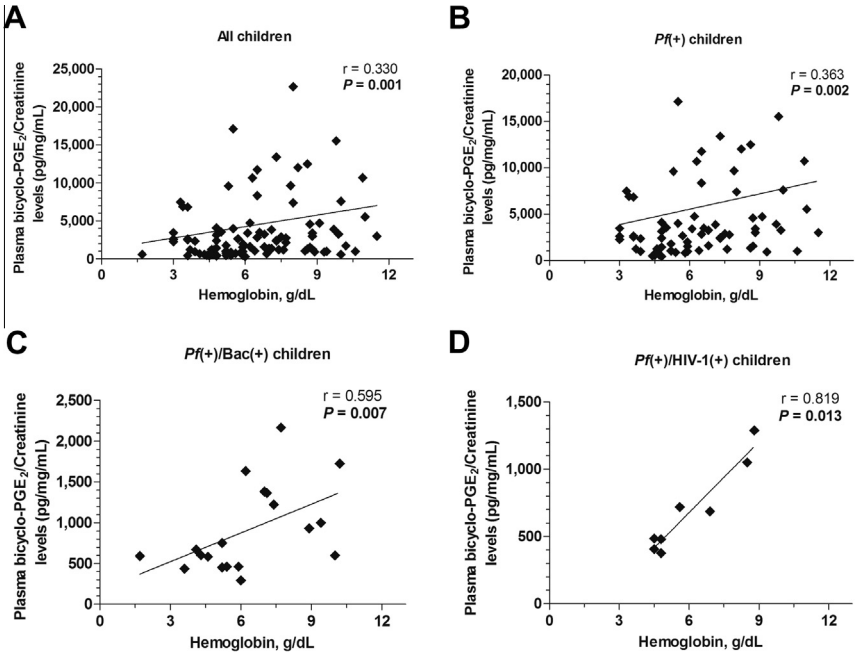

Fig. 2. Relationship between plasma bicyclo-PGE_2/creatinine concentration and Hb levels in children with malaria and co-infection with either bacteremia or HIV-1. Correlation between plasma bicyclo-PGE_2/creatinine and Hb levels in the (A) overall cohort ($n = 101$), (B) falciparum malaria alone [*Pf*(+)] ($n = 74$), (C) malaria plus bacteremia [*Pf*(+)/Bac(+)] ($n = 19$), (D) malaria plus HIV-1 [*Pf*(+)/HIV-1(+)] ($n = 8$). The relationship between plasma bicyclo-PGE_2/creatinine and Hb levels were computed using Spearman's rank correlation analyses.

($r = -0.047$, $P = 0.716$), IFN- γ ($r = -0.114$, $P = 0.265$), and IL-10 ($r = 0.192$, $P = 0.134$). Since a multiplex was performed, additional exploratory analyses were performed to identify potential relationships between COX-2 and regulatory cytokines (Table 2). Only

Table 2
Inflammatory mediator profiles.

Characteristics	*Pf*(+)	*Pf*(+)/Bac(+)	*Pf*(+)/HIV-1(+)	*P*
Sample size (*n*)	74	19	8	
Tumor necrosis factor-alpha (TNF-α), pg/mL	13.8 (53.7)	11.8 (20.0)	56.6 (66.9)	0.126
Interferon-alpha (IFN-α), pg/mL	25.3 (43.0)	77.0 (79.0)	26.4 (26.0)	0.009
Interferon-gamma (IFN-γ), pg/mL	11.4 (24.8)	18.2 (23.7)	7.9 (21.9)	0.056
Monocyte chemoattractant protein (MCP)-1, pg/mL	184.7 (203.7)	446.5 (502.4)	495.9 (843.4)	0.001
Interleukin (IL)-1Ra, pg/mL	1794.2 (3615.5)	4399.7 (18,146.0)	5183.6 (7194.9)	0.045
Interleukin (IL)-4, pg/mL	5.2 (10.0)	22.7 (49.7)	14.5 (15.2)	0.001
Interleukin (IL)-7, pg/mL	28.5 (41.2)	88.6 (89.9)	25.6 (24.4)	<0.001
Interleukin (IL)-8, pg/mL	13.9 (17.0)	22.2 (29.4)	20.7 (17.3)	0.002
Interleukin (IL)-10, pg/mL	233.4 (793.6)	165.5 (211.2)	273.7 (805.3)	0.393
Interleukin (IL)-12p40/70, pg/mL	335.9 (344.1)	660.5 (649.6)	691.8 (509.6)	0.004
Interleukin (IL)-15, pg/mL	25.8 (25.3)	199.1 (222.4)	47.7 (105.3)	<0.001
Interleukin (IL)-17, pg/mL	20.4 (3.0)	39.2 (60.9)	20.4 (0.7)	0.004

Data presented as median (interquartile range, IQR). Children were stratified into those with malaria [*Pf*(+)] alone, malaria plus bacteremia [*Pf*(+)/Bac(+)], and malaria plus HIV-1 [*Pf*(+)/HIV-1(+)], all with any density parasitemia. Kruskal–Wallis test was used to compare differences across groups.

those found to be significant are presented here. There was a general trend of increasing levels of circulating IL-1 receptor antagonist (RA) ($P = 0.045$), IL-8 ($P = 0.002$), IL-12p40/70 ($P = 0.004$), and monocyte chemoattractant protein (MCP)-1 ($P = 0.001$) in co-infected children. Children co-infected with malaria and bacteremia had elevated levels of IFN-α ($P = 0.009$), IL-4 ($P = 0.001$), IL-7 ($P < 0.001$), IL-15 ($P < 0.001$), and IL-17 ($P = 0.004$). Correlation analyses showed an inverse relationship between bicyclo-PGE_2/creatinine and IL-4 ($r = -0.287$, $P = 0.024$), IL-8 ($r = -0.472$, $P < 0.001$) and MCP-1 ($r = -0.389$, $P = 0.002$), with no other analyses revealing a significant association.

4. Discussion

Here, we report for the first time the profiles of circulating PGE_2 levels in children with malaria co-infected with either bacteremia or HIV-1. Results presented here suggest that co-infection further suppresses the already reduced levels of COX-2-derived PGE_2 present in children with malarial anemia [13,15]. Although we have previously shown that COX-2-derived PGE_2 production is suppressed during severe malaria infections [13,14], this is the first report showing the influence of co-infection on the COX-2-PGE_2 pathway. Based on the consistent findings showing decreased PGE_2 in the context of enhanced disease severity in children [12,13,15] and pregnant women [14] with falciparum malaria, adults with vivax malaria [33], experimental models of murine malaria [34–36], and now in co-infected children with malarial anemia, measurement of PGE_2 as a biomarker for severe malaria appears well justified.

We have previously shown in a larger cohort of children with malaria that both bacteremia and HIV-1 enhance the severity of pediatric anemia [5,7,10]. Based on the sample size in the current study in which bicyclo-PGE_2/creatinine and the inflammatory mediators were available in only a subset of the larger population, logistic regression analyses were not performed. However, the progressive worsening of anemia in co-infected children, although non-significant, likely due to sample size issues, suggests that lowered bicyclo-PGE_2/creatinine is indeed associated with enhanced anemia. This premise is supported by the significant positive relationship between circulating bicyclo-PGE_2/creatinine and Hb in both mono- and co-infected children. Data showing that PGE_2 is an important soluble factor for promoting efficient erythropoiesis [37–39], erythroid maturation, and Hb formation [40–44] lends further support to this hypothesis.

A number of previous studies have shown that PGE_2 levels are elevated in HIV-1 [45–48] and HIV-1 and human papilloma virus co-infection [49], possibly accounting for the immunosuppressive effects witnessed in these virally infected patients. In addition, studies in primary human macrophages showed that the HIV-1 Tat protein increased COX-2 expression and PGE_2 synthesis, and that a COX-2 inhibitor, as well as exogenous PGE_2, promoted increased growth of Leishmania [50]. Although the reasons for a different trend reported here, in which COX-2 and PGE_2 are suppressed in children co-infected with malaria and HIV-1, is unclear, it may be related to our previous findings demonstrating that malarial products ingested by circulating phagocytic cells suppress COX-2 mRNA and protein, and the subsequent production of PGE_2, via up-regulation of IL-10 [51]. Thus, it appears that the suppressive effects that malaria has on the COX-2-PGE_2 pathway cannot be overcome when infected with HIV-1, as evidenced by the even greater suppression during malaria and HIV-1 co-infection.

Decreased COX-2 expression and peripheral bicyclo-PGE_2 in children with malaria and bacteremia co-infection parallels results reported in patients with sepsis, in which decreased PGE_2 was associated with enhanced clinical severity [52]. Moreover, this investigation also revealed that the inducibility of COX-2-derived PGE_2 in *in vitro* whole blood stimulation assays was markedly reduced in patients with severe sepsis [52]. Based on these results, the authors suggested that arachidonic acid metabolites were a valid biomarker for disease severity and clinical outcomes in patients with sepsis, an identical justification we propose in the context of malaria.

Although the inflammatory mediators typically associated with regulation of COX-2-derived PGE_2 during malarial infections (e.g., TNF-α, IFN-γ, and IL-10) [12,51,53] were not significantly associated with bicyclo-PGE_2 levels, there was a general trend towards increasing levels of inflammatory mediator production in co-infected children. In addition, there was a significant inverse correlation between bicyclo-PGE_2, and IL-4, IL-8, and MCP-1. Additional analyses in a larger cohort of individuals will be required to determine if the observed relationship between PGE_2, IL-8, and MCP-1 represents an important, yet unexplored, immunological network that influences disease outcomes in mono- and co-infected children.

Author contributions

SBA, PK, and GCD performed the experiments, JBH assisted in the data analysis, DJP, JMO, and JMV designed and executed the study. SBA and DJP co-wrote the manuscript. The authors declare no conflict of interest.

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