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# Possible role of $PGD_2$ in malaria infections

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ABSTRACT

**Objective:** To preliminarily investigate the possible role of prostaglandin $D_2$ ($PGD_2$) in malaria infections.

**Methods:** Blood and urinary samples ($n$ = 120 each) were collected from Thai patients with *Plasmodium falciparum* (*P. falciparum*) with moderate ($n$ = 26) and high ($n$ = 4) parasitemia, patients with *Plasmodium vivax* (*P. vivax*) ($n$ = 30), patients with fever associated with other infections ($n$ = 30), and healthy subjects ($n$ = 30). $PGD_2$ concentrations in plasma and urinary samples of healthy subjects, patients with fever associated with other infections and patients with malaria were determined using Prostaglandin $D_2$-MOX express EIA kit (Cayman Chemical, USA).

**Results:** The possible association between $PGD_2$ and malaria infections is clearly demonstrated with $PGD_2$ concentration in urine. The urinary $PGD_2$ concentrations were relatively high (about 5-fold) in patients with *P. falciparum* with moderate parasitemia and *P. vivax* infections compared with other groups. Furthermore, the concentration in patients with *P. falciparum* with moderate parasitemia and *P. vivax* infection were significantly higher than that in healthy subjects and patients with fever associated with other infections.

**Conclusions:** Urinary $PGD_2$ concentrations may offer a more dependable and useful tool for predicting malaria severity. Confirmation is this preliminary finding is required with a larger sample size.

## 1. Introduction

Globally, an estimated 3.2 billion people in 97 countries and territories are at risk of being infected with malaria and developing disease and 1.2 billion are at high risk. According to the latest estimates, 198 million cases of malaria occurred globally in 2013 and the disease led to 584 000 deaths, representing a decrease in malaria case incidence and mortality rates of 30% and 47% since 2 000, respectively [1]. Among all human malaria species, *Plasmodium falciparum* (*P. falciparum*) is the most severe form with regard to morbidity and mortality. Several factors associated with pathogenesis and severity of severe *P. falciparum* have been reported, but major factors involve the production of cytokines (IL-4, IL-12) and tumor necrosis factor (TNF) [2,3]. Recently, the hypothetical role of hemeoxygenase-1 (HO-1) enzyme in pathogenesis of severe and cerebral malaria has been proposed as one of the important factors that may be linked with susceptibility and severity of malaria infections [4]. HO-1 is the enzyme involved in heme breakdown process to release iron, carbon monoxide, and biliverdin/bilirubin. This process therefore influences iron supply that support the growth of *P. falciparum* [5]. The expression of HO-1 is induced by several substances particularly prostaglandin $D_2$ ($PGD_2$). $PGD_2$ is the most important prostanoid produced in the brain that regulates sleep and pain responses [5]. The production of $PGD_2$ is induced through transcriptional

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activation of cyclooxygenase-2, as well as heme degradation [6]. It is thought that intra-erythrocytic *P. falciparum* parasites release *Pf*$PGD_2$ which may influence heme catabolism in the host cells near the parasite sequestration sites. The sequestered parasitized erythrocytes then generate hemodynamic stress, which in turn, increase the production of *Pf*$PGD_2$ through induction of the expression of lipocalin-type prostaglandin D2 synthase in vascular endothelial cells as reported in the case of fluid shear stress [7]. The aim of the present study was to preliminarily investigate the possible role of $PGD_2$ in malaria infections through measuring its concentrations in blood and urinary samples collected from Thai patients with *P. falciparum*, *Plasmodium vivax* (*P. vivax*), patients with fever, and healthy subjects of both genders and all age groups.

## 2. Material and methods

### 2.1. Study areas and sample collection

The study was conducted at Mae Sot General Hospital, Mae Sot, Tak Province, Thailand. Approval of the study protocol was obtained from the Ethics Committees of Ministry of Public Health of Thailand. A total of 120 blood and urine samples were collected (before treatment) from Thai patients with *P. vivax* ($n$ = 30), patients with *P. falciparum* [$n$ = 26 and 4 for moderate (1 000–50 000 asexual parasite/μL), and high parasitemia (>50 000 asexual parasite/μL), respectively)], patients with fever associated with other infections ($n$ = 30), and healthy subjects ($n$ = 30). Written informed consents for study participation were obtained from all participants [89 males and 31 females, aged (12–90) years] before study.

Blood sample (3 mL) was collected from each participant and serum and plasma (with EDTA anticoagulant and 10 μM indomethacin) samples were prepared through centrifugation at 1 500 × $g$ for 15 min (4 °C). Random mid-stream urine sample (2 mL) was collected and immediately stored at −80 °C without pretreatment with any preservative.

### 2.2. Sample extraction

Plasma sample was diluted with cold acetone at the ratio of 1:1 (v:v) and incubated on ice for 5 min. The precipitated protein was removed through centrifugation at 8 000 × $g$ for 10 min and stored at −20 °C until analysis. Before analysis, the sample was evaporated to dryness using Centri-vap cold trap and the dried sample was resuspended in 100 μL of EIA buffer. Methoximation was performed using Prostaglandin $D_2$-MOX express EIA kit according to the procedure provided by the manufacturer. Concentrations of bilirubin (direct and total) in serum samples were determined immediately after sample collection by diazonium salt 3,5-dichlorophenyldiazonium tetrafluoroborate (DPD) method. Creatinine concentrations in urine samples were determined using Jaffe's reaction method.

### 2.3. Determination of $PGD_2$ concentrations

$PGD_2$ concentrations in plasma and urine samples were determined using Prostaglandin $D_2$-MOX express EIA kit (Cayman Chemical, USA). Briefly, 50 μL of plasma (1:10 dilution) or urine (1:5 dilution) was added in a 96-well plate coated with goat anti-rabbit IgG antibodies. The tracer (50 μL) and the $PGD_2$ specific antibody (50 μL) were added to each well. The plate was incubated overnight at 4 °C and washed five times with 10 mM phosphate buffer (pH 7.4) containing Tween 20 (0.05%) pH 7.4. Two-hundred μL of Ellman's reagent [69 mM acetylthiocholine and 54 mM 5,50-dithio-bis (2-nitrobenzoic acid) in 10 mM phosphate buffer pH 7.4] was added to each well and the plate was incubated in a dark room at 25 °C for (60–90) min. Concentration of the reaction product (yellow solution) was spectroscopically measured using a microplate reader at the wavelength of 410 nm. A standard curve was developed using computer spreadsheet of Cayman Chemical Company and concentrations of $PGD_2$ in plasma and urine samples relative to those standards were determined.

### 2.4. Statistical analysis

Difference in $PGD_2$ concentrations in samples of all groups were determined using Kruskal–Wallis test followed by Mann–Whitney $U$ test for data not conforming to normal distribution. Statistical significance level was set at $\alpha = 0.05$ for all tests.

## 3. Results

### 3.1. Association between plasma $PGD_2$ concentrations and malaria infections

Median (range) values of plasma $PGD_2$ concentrations in samples collected from patients with *P. vivax*, patients with *P. falciparum* (moderate and high parasitemia), patients with fever associated with other infections, and healthy subjects are summarized in Table 1. The concentration was highest (about 3-fold) in patients with fever associated with other infections compared with other groups. The concentration in *P. falciparum* infection with moderate parasitemia was significantly higher than healthy subjects ($P < 0.000\,1$), but significantly lower than *P. vivax* infection ($P < 0.000\,1$) and patients with fever associated with other infections ($P < 0.000\,1$). For *P. vivax* infection, the concentration was significantly higher than patients with *P. falciparum* with moderate parasitemia ($P < 0.000\,1$) and healthy subjects ($P < 0.000\,1$), but significantly lower than patients with fever associated with other infections ($P < 0.05$).

### 3.2. Association between serum bilirubin concentrations and malaria infections

Median (range) values of serum concentrations of bilirubin (direct and total) in samples collected from all groups are summarized in Table 1. The concentrations in patients with *P. falciparum* with moderate parasitemia and *P. vivax* infections were significantly higher than healthy subjects ($P < 0.05$ and $P < 0.05$, respectively). The increased bilirubin level did not interfere the measurement of serum $PGD_2$ level.

The median (range) direct bilirubin concentrations in patients with *P. falciparum* with moderate parasitemia and *P. vivax* infections were significantly higher than healthy subjects ($P < 0.05$ and $P < 0.05$, respectively). In addition, the concentration in patients with fever associated with other infections was significantly higher than healthy subjects ($P < 0.05$).

**Table 1**
Median (range) plasma concentrations of $PGD_2$, serum total bilirubin concentration, serum direct bilirubin concentration, urinary $PGD_2$ concentration and urinary creatinine concentration in 4 groups.

| Group | Number of sample | Plasma $PGD_2$ concentration (pg/mL) | Serum total bilirubin concentration (mg/dL) | Serum direct bilirubin concentration (mg/dL) | Urinary $PGD_2$ concentration (pg/mL) | Urinary creatinine concentration (mg/dL) |
|---|---|---|---|---|---|---|
| Control Group | 30 | 16 (6–30)[a,b,c] | 0.50 (0.26–1.10)[e,f] | 0.27 (0.02–0.51)[d,e,f] | 26 (7–41)[d,f] | 106.16 (20.80–267.73)[e,f] |
| Group A | 30 | 60 (11–525)[b,f,g] | 0.57 (0.12–29.26) | 0.33 (0.09–16.25)[h] | 23 (7–120)[e,f] | 75.35 (26.90–303.60)[e,f,h] |
| Group B | 26 | 22 (13–75)[a,c,g] | 1.11 (0.38–2.78)[f,h] | 0.49 (0.17–1.03)[d,h] | 120 (29–325)[d,h] | 188.80 (61.90–378.30)[d,h] |
| Group C | 4 | 28 (16–38) | 1.24 (0.74–1.74) | 0.66 (0.30–1.03) | 56 (49–260) | 203.90 (151.50–274.60) |
| Group D | 30 | 34 (22–130)[b,d,g] | 0.91 (0.33–2.74)[e,h] | 0.43 (0.18–1.70)[h] | 139 (23–1 123)[d,h] | 183.75 (47.80–349.20)[d,h] |

Control Group: Healthy subjects. Group A: Patients with fever associated with other infections; Group B: *P. falciparum* with moderate parasitemia; Group C: *P. falciparum* with high parasitemia; Group D: *P. vivax*.
[a] $P < 0.000\,1$ compared with Group A. [b] $P < 0.000\,1$ compared with Group B. [c] $P < 0.000\,1$ compared with Group D. [d] $P < 0.05$ compared with Group A. [e] $P < 0.05$ compared with Group B. [f] $P < 0.05$ compared with Group D. [g] $P < 0.000\,1$ compared with Control Group. [h] $P < 0.05$ compared with Control Group.

### *3.3. Association between urinary $PGD_2$ concentrations and malaria infections*

Median (range) values of urinary $PGD_2$ concentrations in plasma samples collected from all groups are summarized in Table 1. The median urinary $PGD_2$ concentrations were relatively high (about 5-fold) in patients with *P. falciparum* with moderate parasitemia and *P. vivax* infections compared with other groups. The concentration in patients with *P. falciparum* with moderate parasitemia was significantly higher than that in healthy subjects ($P < 0.05$) and patients with fever associated with other infections ($P < 0.05$). For *P. vivax* infection, the concentration was significantly higher than that in healthy subjects ($P < 0.05$) and patients with fever associated with other infections ($P < 0.05$).

### *3.4. Association between urinary creatinine concentrations in urine samples and malaria infections*

Median (range) values of urinary creatinine concentrations in samples collected from all groups are summarized in Table 1. The concentration in patients with *P. falciparum* with moderate parasitemia was significantly higher than healthy subjects ($P < 0.05$) and patients with fever associated with other infections ($P < 0.05$). For *P. vivax* infection, the concentration was significantly higher than healthy subjects ($P < 0.05$) and patients with fever associated with other infections ($P < 0.05$).

## 4. Discussion

$PGD_2$ is markedly produced in the human brain to control sleep and pain responses. $PGD_2$ is also synthesized in mast cells and leukocytes by a cellular, myeloid-type, glutathione-dependent $PGD_2$ synthase [7]. *Pf*$PGD_2$ is a potential factor derived from intra-erythrocyte falciparum parasites. In a previous study in human astrocyte cell line (CCF-STTG1), $PGD_2$ increased the expression of HO-1 mRNA in a dose- and time-dependent manner. Therefore, $PGD_2$ might be involved in the pathogenesis of cerebral malaria through the induction of HO-1 expression in malaria patients [5]. In another study in human retinal pigment epithelial cells (ARPE-19, D407), $PGD_2$ was shown to stimulate the expression of HO-1 mRNA and protein through binding to prostaglandin $D_2$ receptor ($DP_2$), linking the $PGD_2$-$DP_2$ with heme homeostasis [8]. Study in Gambian children with severe malaria demonstrated that (GT) ($n$) repeat polymorphism in the HMOX1 promoter influenced the magnitude of HO-1 gene expression, while high HO-1 level was associated with severe disease [4]. The present study aimed to investigate the possible link between $PGD_2$ concentrations in plasma and urinary samples and malaria infections, through the induction of HO-1 enzyme. Plasma $PGD_2$ concentration was shown to be significantly higher in patients with *P. falciparum* infection with moderate and high parasitemia compared with healthy subjects. It was noted however that, $PGD_2$ concentrations in patients with *P. vivax* infection and those with fever associated with other infections were respectively, about 2- and 4-fold of that in healthy subjects. The variability of the $PGD_2$ concentrations measured could be due to interference from other substances in plasma samples particularly bilirubin. Significantly higher serum total and direct bilirubin concentrations were observed in samples collected from malaria patients compared with healthy subjects. Moreover, $PGD_2$-EIA anti-serum derived from human $PGD_2$ used in the EIA method might be non-specific to *Pf*$PGD_2$. This may suggest the difference between human $PGD_2$ and *Pf*$PGD_2$ [9].

The possible association between $PGD_2$ and malaria infections is clearly demonstrated with $PGD_2$ concentration in urine. The urinary $PGD_2$ concentrations were relatively high (about 5-fold) in patients with *P. falciparum* with moderate parasitemia and *P. vivax* infections compared with other groups. Furthermore, the concentration in patients with *P. falciparum* with moderate parasitemia and *P. vivax* infection were significantly higher than that in healthy subjects and patients with fever associated with other infections. Urinary $PGD_2$ concentrations may offer a more dependable and useful tool for predicting malaria severity. Plasma $PGD_2$ is not a suitable matrix due to rapid degradation of $PGD_2$ in the presence of plasma protein especially albumin, which complicates the analysis of $PGD_2$ [10]. For determination of $PGD_2$ in plasma as well as tissue homogenates, samples needs to be extracted immediately after collection to remove proteins and to stabilize $PGD_2$. Indomethacin was immediately added to whole blood sample after collection to prevent *ex vivo* formation of eicosanoids which have the potential to interfere with the EIA assay. For urinary samples however, the addition of indomethacin is not required [7]. Further study in a larger sample size should be performed to confirm the possible association between $PGD_2$

levels and malaria infections including its predicting tool for malaria disease severity. One limitation of the study is that spot urine was used to measure the $PGD_2$ levels. The spot urine can be diluted or concentrated depending on patients' urine volume and renal function.

## Conflict of interest statement

We declare that we have no conflict of interest.

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