

# Relationship of hepcidin with parasitemia and anemia among patients with acute, uncomplicated *Plasmodium falciparum* malaria in Ghana

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## Abstract

The pathogenesis of malarial anemia is incompletely understood. Hepcidin, a recently discovered peptide hormone, is a major regulator of iron metabolism and is thought to play a central role in the anemia of chronic inflammation. The specific aim of the study was to characterize the association between urinary hepcidin, hemoglobin, and parasitemia in 199 patients with acute *Plasmodium falciparum* malaria in Ghana. Urinary hepcidin was measured using surface-enhanced laser desorption/ionization time-of-flight mass spectrometry. Urinary hepcidin (intensity/mmol creatinine) was associated with log parasitemia in 86 children (beta = 0.086, standard error [SE] = 0.035,  $P < 0.017$ ), 31 pregnant women (beta = 0.218, SE = 0.085,  $P < 0.016$ ), and 82 adults (beta = 0.184, SE = 0.043,  $P < 0.0001$ ). Urinary hepcidin was not significantly associated with hemoglobin or anemia. Urinary hepcidin is more strongly associated with parasitemia than hemoglobin or anemia among patients with acute *P. falciparum* malaria in Ghana.

## Methods

The study subjects consisted of a consecutive sample of 290 individuals (187 adults, including 58 pregnant women, and 103 children) seen at Kpone Health Centre in Kpone On Sea, Ghana, from July to August, 2005. The Kpone Health Centre serves Kpone On Sea, a village in eastern coastal Ghana, an area that is endemic for *Plasmodium falciparum* malaria. Thick and thin Giemsa-stained blood films were analyzed for the number of parasites per 200 white blood cells. Urinary hepcidin was measured using surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS).<sup>6</sup> A peak is recorded for hepcidin at the characteristic m/v of 2790 when the signal-to-noise ratio is  $>3:1$ . Urinary creatinine was measured using a commercial ELISA (Quidel Corporation, San Diego, CA). Urinary hepcidin concentrations were expressed as intensity per mmol/L creatinine. Anemia was defined as hemoglobin  $<11$  g/dL for children and pregnant women,  $<12$  g/dL for non-pregnant adult women, and  $<13$  g/dL for men.<sup>7</sup>

## Results

Of the 290 subjects in the study series, urine samples were available from 199 patients (86 children, 82 adults, and 31 pregnant women) with acute, uncomplicated *Plasmodium falciparum* malaria. The prevalence of anemia among children, adults, and pregnant women was 79.1%, 82.9%, and 90.3%, respectively. Linear regression models were used to examine the relationship between urinary hepcidin and parasitemia, hemoglobin, and anemia in each of the three groups and in all three groups combined (Table 1). Urinary hepcidin was associated with parasitemia in children, adults, and pregnant women, and in all three groups combined. Hepcidin was not significantly associated with hemoglobin or anemia in any of the groups. In a multivariate linear regression model, log<sub>10</sub> urinary hepcidin was associated with log<sub>10</sub> parasitemia (beta = 0.174, SE = 0.026,  $P < 0.0001$ ) but not hemoglobin (beta = 0.022, SE = 0.058,  $P = 0.71$ ). There were no significant interactions between log<sub>10</sub> parasitemia and hemoglobin.

## Regulation of Hepcidin

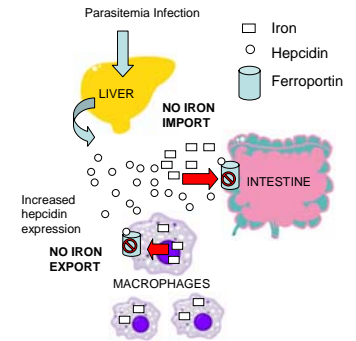
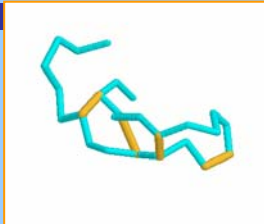


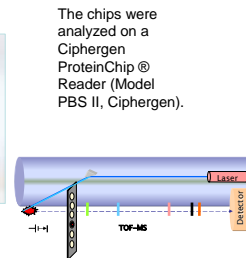
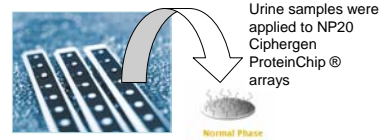
Figure 1: Regulation of hepcidin in anemia of infection. Increased hepcidin expression inhibits the release of iron from stores by binding to the iron-exporter ferroportin, inducing its internalization and degradation.<sup>12</sup>

Images from weebis.org and wikipedia.org

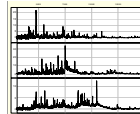
## Hepcidin-25



## ProteinChip® with SELDI-TOF



The chips were analyzed on a Ciphergen ProteinChip® Reader (Model PBS II, Ciphergen). Peak annotation was conducted using Ciphergen ProteinChip Software (version 3.2.0), after baseline subtraction and adjustment.



Images provided by Ciphergen®

## Table 1

Univariate linear regression models of hepcidin and other risk factors in each group and combined

Characteristic	Beta	SE	P
<b>Children</b>			
Log parasitemia	0.086	0.035	0.017
Hemoglobin	0.048	0.086	0.58
Anemia	0.345	0.388	0.38
<b>Adults</b>			
Log parasitemia	0.184	0.043	$<0.0001$
Hemoglobin	-0.030	0.116	0.79
Anemia	0.900	0.487	0.07
<b>Pregnant Women</b>			
Log parasitemia	0.218	0.085	0.016
Hemoglobin	-0.110	0.193	0.54
Anemia	0.677	1.083	0.54
<b>All Groups Combined</b>			
Log parasitemia	0.171	0.025	$<0.0001$
Hemoglobin	-0.101	0.061	0.10
Anemia	0.481	0.317	0.13

## Discussion

The present study provides some initial insight into the pathogenesis of anemia during malaria. High levels of parasitemia were associated with high levels of hepcidin. Hepcidin inhibits duodenal iron absorption at the intestinal epithelium<sup>11</sup> and inhibits mobilization of iron from the liver and spleen.<sup>2</sup> Hepcidin binds to the iron exporter, ferroportin, inducing its internalization and degradation.<sup>12</sup> Ferroportin is the only mammalian iron exporter identified to date and is necessary for maternofoetal iron transfer and iron efflux from duodenal enterocytes, macrophages, and hepatocytes.<sup>13</sup> The finding from the present study suggests that during acute, uncomplicated malaria, the availability of iron for erythropoiesis may be limited through the upregulation of hepcidin.

## Introduction

Malaria is a leading cause of morbidity and mortality and accounts for an estimated 500 million cases and 1-3 million deaths annually in developing countries.<sup>1</sup> Anemia is one of the most common and severe outcomes of *Plasmodium falciparum* malaria, and its pathogenesis is incompletely understood. Hepcidin, a recently discovered peptide hormone synthesized primarily by hepatocytes, is considered a major regulator of iron homeostasis.<sup>2</sup> Hepcidin regulates iron metabolism by inhibiting duodenal iron absorption at the intestinal epithelium and by affecting mobilization of iron from liver and spleen.<sup>2</sup> The synthesis of hepcidin is modulated by a more iron-replete state, hypoxia, and inflammation.<sup>3,5</sup> In the anemia of chronic inflammation, hepcidin is thought to block the release of iron from enterocytes, hepatocytes, and macrophages, leading to hypoferrremia and limited iron availability for erythropoiesis.<sup>2</sup> Although studies suggest that hepcidin is a key regulator in the anemia of chronic inflammation, the role of hepcidin in the pathogenesis of malarial anemia has not been characterized. We hypothesized that urinary hepcidin levels would be associated with high parasitemia among patients with acute, uncomplicated *P. falciparum* malaria. To address this hypothesis, we measured urinary hepcidin among patients with malaria in Ghana.

## Conclusion

Hepcidin expression is upregulated by hypoxia,<sup>4</sup> but in the present study, low hemoglobin levels or anemia was not associated with urinary hepcidin. In multivariate analyses, parasitemia was the strongest factor associated with hepcidin, suggesting that parasitemia, and possibly inflammation associated with parasitemia, are stronger modulators of hepcidin expression than hypoxia.

## Acknowledgements

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