

## Plasma chitotriosidase activity in acute *Plasmodium falciparum* malaria

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Received 7 August 2002; received in revised form 30 January 2003; accepted 6 February 2003

### Abstract

**Background:** Chitotriosidase is a functional chitinase secreted by activated macrophages. It is encoded by a gene located on chromosome 1q31-32, whose mutations may be responsible for chitotriosidase deficiency, encountered in almost 6% of Caucasian population. **Objective:** This study reports firstly plasma chitotriosidase activity in African children with acute *Plasmodium falciparum* malaria. The chitotriosidase activity was correlated to objective parameters reflecting the status of the disease and compared with those found in healthy African children. **Results:** We found that plasma chitotriosidase levels are significantly increased in African children with acute malaria ( $185.0 \pm 141.0$  nmol/h/ml; median 150; range 11–521) with respect to reference values obtained in age matched African children ( $84.45 \pm 72.8$  nmol/ml/h; median 63; range 4–350) ( $P < 0.001$ ). Moreover the levels of chitotriosidase were higher in African children than in Caucasian children matched for age ( $28.86 \pm 18.7$  nmol/h/ml; median 24; range 1–98) ( $P < 0.0001$ ). A remarkable significant correlation was found between plasma chitotriosidase and reticulo-endothelial activation, as judged by thrombocytopenia degree and serum ferritin level in children with acute malaria. **Conclusion:** Based on this study, it appears that genetic and environmental features might be responsible for diversity of plasma chitotriosidase activity in black children living in Burkina Faso.

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**Keywords:** Plasma chitotriosidase; *Plasmodium falciparum* malaria; Macrophage

### 1. Introduction

Malaria is a major cause of morbidity and mortality in tropical countries. *Plasmodium falciparum* malaria is the most lethal form and is increasing both in incidence and in its resistance to antimalarial agents [1,2]. A variety of abnormalities in the number, morphology, and function of blood and bone

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marrow cells may be found in *P. falciparum* and *Plasmodium vivax* malaria. The nature of such abnormalities may depend on the pattern and intensity of malaria transmission in the affected area and on the extent of host immunity [3]. Moreover, there are individual factors, which have been recognized to strongly influence the clinical expression [4], which include T-cell-derived cytokines production in response to the infection, macrophage activation and hyperplasia.

The role of macrophage-derived factors [5], such as tumour necrosis factor (TNF)-alpha, in the pathogenesis of haematological abnormalities and macrophage dysfunction in malarial infection has long been known [6]. Chitotriosidase is a functional chitinase produced by activated macrophages [7]: a striking increase of plasma chitotriosidase, a macrophage marker, has been first identified in patients with Gaucher disease [8], a lysosomal disease due to the deficiency of  $\beta$ -glucocerebrosidase enzyme [9]; a slight to moderate increase of chitotriosidase has been reported also in various other lysosomal storage disorders and hematological and infectious diseases involving activated macrophages [10,11]. On the contrary chitotriosidase deficiency has been encountered in almost 6% of Caucasian population [7], resulting in a homozygous 24-base pair duplication in exon 10, generating a mRNA with an in-frame deletion of 87 nucleotides [12].

In this study plasma chitotriosidase activity was firstly measured in African children affected by acute malaria infection and it was correlated to objective parameters reflecting the status of the disease.

## 2. Materials and methods

### 2.1. Study area

The study took place between July and October 2000 in Ouagadougou (Burkina Faso-Africa); *P. falciparum* malaria is a major cause of morbidity and mortality in children in this region with most transmission occurring during and shortly after the rainy season from June to October. Children with malaria and control subjects were evaluated and enrolled at the local Centre Medical Saint Camille (CMSC). Ethical

approval for the study was received from the institutional review board at the CMSC.

### 2.2. Subjects

Data on personal characteristics (age, sex), clinical findings (history, symptoms, temperature) and parasitological data (thin and thick blood films) were collected from all the participants to the study.

– Sixty-seven patients affected by acute malaria were diagnosed in the study period; the patients comprise 40 males and 27 females, aged 2–72 months (median age: 16 months). Blood smears were prepared and examined by a single, trained individual. The level of parasitemia was determined by counting the number of parasites per 100 red cells in a thick film and it was graded from 1+ to 4+ corresponding to 1 to > 3 parasites/100 red blood cells respectively. Blood samples were taken for evaluation of hemochrome, serum ferritin and plasma chitotriosidase levels. Clinical symptoms were consistent with “severe malaria” in 39 (58.2%) children, which had highest body temperature, poor general conditions, neurological impairment of variable degree and respiratory difficulties. 28 (41.8%) children had “uncomplicated malaria” with fever, headache, nausea, myalgias or gastrointestinal symptoms without any findings of severe malaria. No correlation was found between the severity of clinical signs and age and gender distribution or the parasitemia degree.

– Control subjects included 147 African children (80 males and 67 females) with age ranging from 4 to 150 months (median 20): at the time of evaluation. Control subjects had no serious underlying illness nor they had hematological disorders (haemoglobinopathies, leishmaniasis) or various infectious diseases known to influence plasma chitotriosidase levels. They had no signs of acute infectious disease and negative smears for *P. falciparum*. Informed consent was obtained before blood sampling from their relatives or guardians. All studied children were negative for HIV antibody testing.

### 2.3. Laboratory procedures

Hematological parameters including hemochrome and serum ferritin were measured by standard laboratory methods. For chitotriosidase assay, 3 ml of

EDTA-blood were centrifuged and plasma samples were stored at  $-20\text{ }^{\circ}\text{C}$  until determination by fluorimetric method [20] at the Center for Metabolic Diseases, University of Catania, Italy. Chitotriosidase was measured by incubating  $5\text{ }\mu\text{l}$  of undiluted plasma with  $100\text{ }\mu\text{l}$  of a solution containing  $22\text{ }\mu\text{mol/l}$  of the artificial substrate 4-methylumbelliferyl- $\beta\text{-D-N,N',N''}$  triacetylchitotriose (Sigma) in  $0.5\text{ mmol/l}$  citrate-phosphate buffer pH 5.2, for 15 min at  $37\text{ }^{\circ}\text{C}$ . The reaction was stopped by using  $2\text{ ml}$  of  $0.5\text{ mol/l}$   $\text{Na}_2\text{CO}_3\text{-NaHCO}_3$  buffer, pH 10.7. The fluorescence was read by a Perkin Elmer fluorimeter, on  $365\text{ nm}$  excitation and  $450\text{ nm}$  emission. Chitotriosidase activity was measured as nanomoles of substrate hydrolyzed per ml per hour (nmol/ml/h). Samples with a chitotriosidase levels  $>100\text{ nmol/ml/h}$  were reassayed after a dilution of 10-fold or 50-fold with distilled water. Reference values were obtained by measuring plasma chitotriosidase in 329 Caucasian healthy children aged 12–70 months.

2.4. Statistical analyses

Demographic, clinical and treatment profiles were recorded on computer file and analyzed by standard software (SPSS-10). We used the two-tailed Student's *t*-test for parametric and  $\chi^2$  test for non-parametric

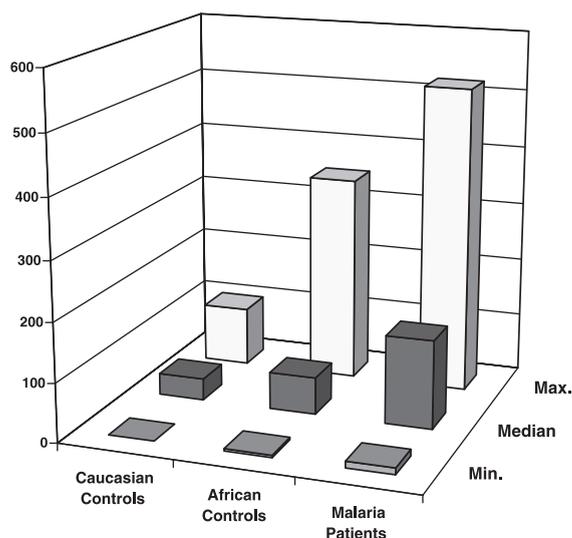


Fig. 1. Plasma chitotriosidase levels (nmol/ml h) in 67 children with acute malaria, 147 African control children and 329 Caucasian healthy children.

Table 1  
Plasma chitotriosidase levels and haematological parameters in African children with acute *P. falciparum* malaria and control subjects

	Plasma chitotriosidase (nmol/ml/h)	Age (months)	Leukocyte ( $\text{mm}^3$ )	Red cells ( $\text{mil}/\text{mm}^3$ )	Hb (g/dl)	Ht (%)	MCV ( $\text{u}^3$ )	MCH (YY)	MCHC (%)	Platelets ( $\text{mm}^3 \times 1000$ )	Serum ferritin (ng/ml)
Malaria children ( $n=67$ )	$185 \pm 141^*$ 150 (11–521)	16 (2–172)	$17.5 \pm 11^*$	$2.1 \pm 1.1^*$	$5.4 \pm 2.6^*$	$16.6 \pm 7.8^*$	$80 \pm 17$	$27 \pm 5.6^*$	$32 \pm 3.8^*$	$186 \pm 118^*$	400 (28–2500)*
Control children ( $n=147$ )	$84.4 \pm 72.8$ 63 (4–350)	20 (4–150)	$8.53 \pm 2.7$	$3.95 \pm 0.78$	$9.6 \pm 2.3$	$26.4 \pm 4.4$	$81.5 \pm 11.9$	$24.5 \pm 4.4$	$30.2 \pm 2.6$	$324.1 \pm 102.2$	24 (3–628)

\*  $P < 0.001$ .

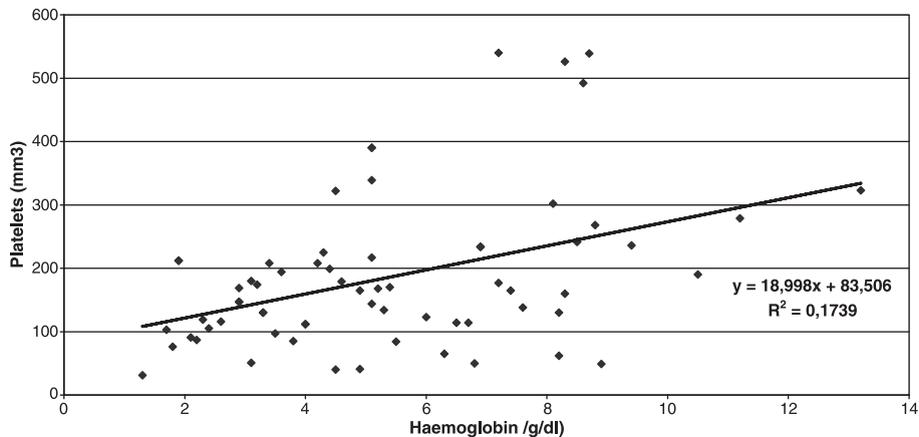


Fig. 2. Correlation between hemoglobin and platelets levels in 67 children with acute *P. falciparum* malaria.

values.  $P$  value  $< 0.01$  was considered statistically significant.

### 3. Results

#### 3.1. Plasma chitotriosidase levels

Chitotriosidase levels in plasma of acute malaria patients, African healthy children without clinical and laboratory findings of malaria infection (control subjects) and Caucasian healthy subjects are shown in Fig. 1.

As a whole, African healthy children and children affected with malaria had significantly increased

mean plasma chitotriosidase levels ( $84.45 \pm 72.8$  and  $185.0 \pm 141.0$  nmol/ml/h, respectively), with respect to reference values obtained in Caucasian subjects ( $28.86 \pm 18.7$  nmol/ml/h) ( $P < 0.0001$ ). Therefore, among Africans, plasma chitotriosidase levels were found elevated and higher values were found in children with acute malaria ( $P < 0.001$ ).

Moreover the number of patients with elevated chitotriosidase levels ( $> 200$  nmol/ml/h) was significantly higher in African children affected with malaria (28/67; 42%) with respect to control African children (18/147; 12.24%) ( $P < 0.0001$ ).

Among malaria patients, we did not encounter subjects with very low ( $< 2$  nmol/ml/h) or undetectable chitotriosidase activity consistent with a diagnosis

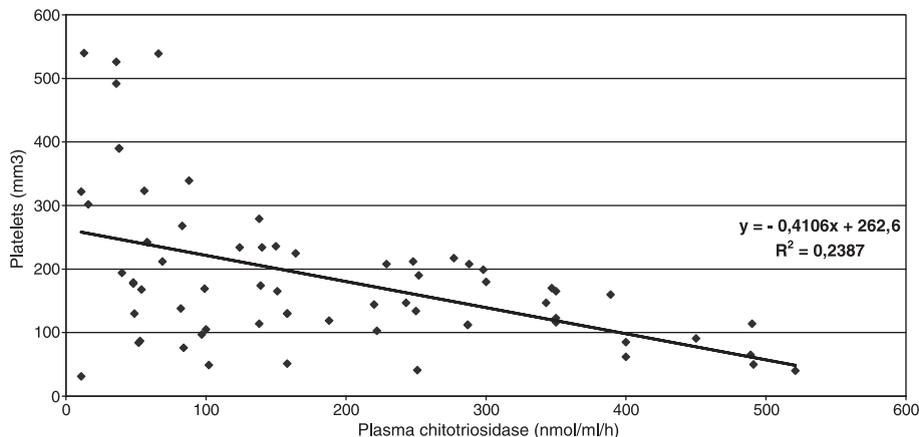


Fig. 3. Correlation between plasma chitotriosidase levels and platelets number in 67 children with acute *P. falciparum* malaria.

of deficiency. None of 147 African control subjects was deficient in chitotriosidase activity while 4/85 (5%) Caucasian subjects had chitotriosidase deficiency ( $P < 0.0001$ ).

### 3.2. Hematological parameters and plasma chitotriosidase

Main hematological parameters in African children with malaria and control African subjects are reported in Table 1. The parasitemia degree was high (level 3–4+) in 27 out of 67 (40%) patients and moderate (level 2–3+) in 40 (60%) patients, respectively.

A significant correlation was found between hemoglobin concentration and platelet number in children with malaria (Fig. 2).

Platelet levels were significantly lower among malaria patients with elevated ( $>200$  ng/ml) plasma chitotriosidase levels (median 134,000/mm<sup>3</sup>; range 40,000–270,000) with respect to malaria patients with normal level ( $<200$  ng/ml) of plasma chitotriosidase (median 227,000/mm<sup>3</sup>; range 50,000–540,000;  $P < 0.001$ ) (Fig. 3). However the number of subjects with low Hb levels ( $<6$  g/dl) was not significantly higher among malaria children who had elevated ( $>200$  ng/ml) chitotriosidase activity in plasma ( $\chi^2 = 1.415$ ;  $P < 0.234$ ).

A significant correlation was found between serum ferritin and plasma chitotriosidase levels (Fig. 4), as

serum ferritin levels were significantly higher in malaria children with increased ( $>200$  ng/ml) plasma chitotriosidase levels (median 823 ng/ml; range: 39–2500) with respect to children with normal ( $<200$  ng/ml) plasma chitotriosidase levels (median 199 ng/ml; range: 28–1500,  $P < 0.0001$ ). Serum ferritin levels were normal or reduced in African subjects without malaria infection, while these were in the normal range among healthy Caucasian subjects.

Plasma chitotriosidase levels were not related to other hematological parameters including red cell and leukocyte count, Ht, MCV, MCH and MCHC nor to the degree of parasitemia.

## 4. Discussion

Chitotriosidase is the first discovered human analogue of chitinases belonging to family 18 of glycosyl hydrolases [7–12]; it is encoded by a gene located on chromosome 1q31–32, whose cDNA is highly homologous to that of chitinases from different species [13]. Chitotriosidase is specifically expressed by phagocytes [14] and is capable of cleaving artificial chitin-like substrate and chitin from fungal cell, suggesting a possible role in defence mechanisms against these microorganisms [14]. Recently, a second mammalian chitinase, named acidic mammalian chitinase (AMCase), has been identified [12]. AMCase enzyme

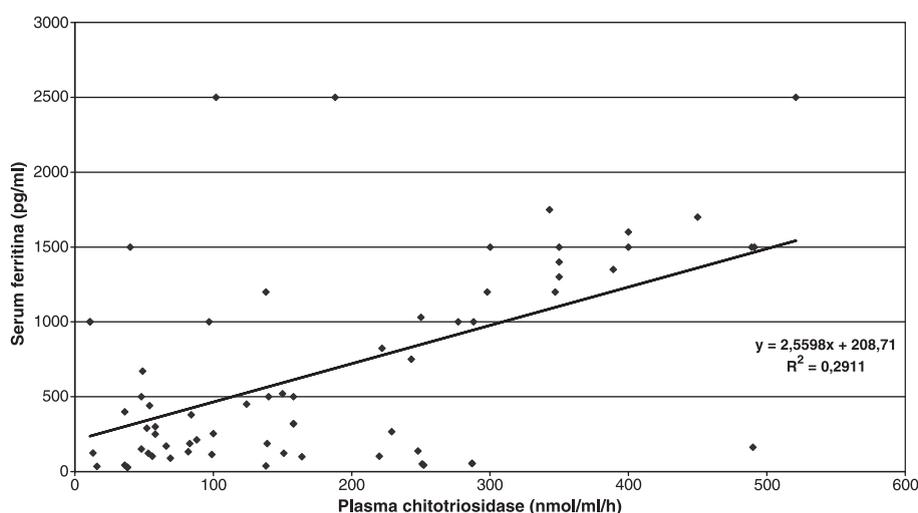


Fig. 4. Correlation between plasma chitotriosidase and serum ferritin levels in 67 children with acute *P. falciparum* malaria.

is characterized by an acidic isoelectric point and extreme stability at acid pH; it is relatively abundant in the gastrointestinal tract and lung, supporting a possible role for chitinases in human defense mechanisms.

In the present study we demonstrated that plasma chitotriosidase levels are medially increased in African children with respect to reference value obtained in age matched Caucasian control subjects. Interestingly, we found that among Africans the number of subjects with elevated chitotriosidase levels was significantly higher in children affected with malaria (42%) with respect to control African children (12.24%). We did not assess the relationship between chitotriosidase and malaria clinical presentation and outcome, rather we assessed the relationship between chitotriosidase and hematological variables which are objective malaria disease parameters. Plasma chitotriosidase levels did not correlate with hemoglobin levels while they were related to thrombocytopenia degree (see Fig. 2).

In addition to ineffective erythropoiesis and inhibition of red cell production [15], the occurrence of anemia in *P. falciparum* malaria has been related to red cell destruction [16], and phagocytosis and hypersplenism [17]. Reduced platelet levels in malaria infection may also be related to activation of the reticulo-endothelial system [4]. Thus, the increase of plasma chitotriosidase in malaria might reflect an activation of the reticulo-endothelial system and storage of membrane glycolipids in the macrophages; however, the lack of correlation between chitotriosidase and Hb levels might be related to the complex origin of malaria induced anemia [15].

We suggest that malaria-induced red cell destruction might have triggered chitotriosidase overproduction by accumulation in macrophages of erythrocyte membrane degradation products and also by enhancing intracellular iron overload. As a matter of fact, the sharp increase of plasma chitotriosidase activity in Gaucher disease might reflect the burden of Gaucher-cells [18], lipid-laden macrophages due to the storage of glycolipids, which are major component of erythrocyte membrane break-down products. We previously demonstrated that patients with  $\beta$ -thalassemia, a genetic defect of  $\beta$ -globin chain synthesis resulting in peripheral anemia, unproductive erythropoiesis and enormous expansion of the reticulo-endothelial system, may have an increase of plasma chitotriosidase, com-

parable to that seen in Gaucher disease [20]. In fact the chitotriosidase enzyme is massively secreted (almost 100-fold) by cultured macrophages that accumulate glycosphingolipid [7,18], and on the other hand, the non-specific stimulation of rat macrophages in vitro brings only to 1.5–2.0-fold increased chitotriosidase activity [19]. Moreover in this study a significant correlation between plasma chitotriosidase and serum ferritin levels in children with acute malaria was found. The possible role of chitotriosidase in iron metabolism is as yet unknown, but it was demonstrated that experimentally induced iron overload in rats leads to an increase of chitotriosidase secretion [21]. The possibility of monitoring macrophage functions through plasma chitotriosidase levels might be useful in malaria affected patients.

The high carrier frequency of the mutated allele of almost 35% of Caucasian population, suggests that chitotriosidase enzyme may become redundant (i.e. not essential for defence mechanisms), in Caucasian with respect to Africans, and it suggests that in Africa, chitotriosidase activity might still play a major role in control of endemic diseases mediated by chitin-containing pathogens including nematodes and fungi. Based on this study, it appears that genetic and environmental features might be responsible for diversity of plasma chitotriosidase activity among different parts of the World.

## Acknowledgements

We thank Mr. Giuseppe Rapicavoli, Department of Pediatrics, University of Catania, for his skilful technical assistance in laboratory work.

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