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Synchronic macrophage response and *Plasmodium falciparum* malaria

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Human Chitotriosidase (CHIT), produced by activated macrophage, is a member of the chitinase family, a group of enzymes with the capability to hydrolyze chitin[1]. Recently plasma CHIT activity was found elevated in children with acute *P. falciparum* malaria compared with healthy African children, as a consequence of macrophage activation due to the presence of parasites[2]. In this study we recruited at the local Centre Medical Saint Camille (CMSC) of Ouagadougou, the capital of Burkina Faso, 62 African children (30 males and 32 females, aged 2–140 months; median 16.5 months), affected by acute *P. falciparum* malaria, born and living in Burkina Faso. Control subjects included 140 healthy African children (79 males and 61 females) with age ranging from 10 to 100 months (median 22 months) at evaluation time. They did not show signs of acute infectious disease and their blood smears for *P. falciparum* were negative. This study was approved by the local Ethical Committees of CMSC. Parents of the participating children in the study were orally informed of the scope of this research. For plasma CHIT assay, 3 ml of EDTA-blood was centrifuged and plasma samples were stored at −40°C until determination by fluorimetric method[3] at the Centre for Metabolic Diseases—University of Catania, Italy.

Patients were classified based on symptoms, physical signs and laboratory findings of malaria at the time of first presentation and diagnosis of malaria was confirmed by microscopic detection of asexual *P. falciparum* in the peripheral blood. According to the WHO criteria, children had "severe malaria", when showing neurological impairment, respiratory distress, oliguria, cardiovascular shock, jaundice and diffuse hemorrhages. In addition, they suffered from severe anaemia (Ht < 15%), parasitaemia degree > 2.5×10^5/µl or > 2.5% in non immune subjects and hypoglycemia (serum glucose less than 2.2 mmol/l corresponding to 40 mg/dl). Mild malaria (uncomplicated malaria) was established by microscopic confirmed parasitaemia degree < 2.5×10^5/µl or < 2.5% with fever, headache, myalgias or gastrointestinal symptoms without any symptoms of severe malaria.

Based on their clinical and haematological parameters, 22 children (35.48%) were affected by severe

malaria, while 40 children (64.51%) were affected by uncomplicated malaria (Table 1).

Medially all children with acute *P. falciparum* malaria had levels of plasma CHIT activity higher (median 140 nmol/ml/h, range 13–521) than healthy controls (median 72 nmol/ml/h, range 14–150; $p < 0.0001$) and the distribution of plasma CHIT activity in these children showed a bimodal behaviour. Also Hb level, red cell count, leukocytes count, platelet count and serum ferritin were found statistically different from the healthy controls ($p < 0.0001$) in all children with acute *P. falciparum* malaria (Table 1).

The children with severe malaria showed haemoglobin levels below 6 g/dl and elevated parasitaemia degree (365,000/µl, range $2.5–5 \times 10^{5)}$. In the children with uncomplicated malaria severe abnormalities of haematological parameters were not encountered (haemoglobin levels >7 – <9 g/dl), while the parasitaemia degree was 250,000/µl below 2.5×10^5/µl. Platelet count was significantly ($p < 0.0001$) lower (141.85 ± 61.94 mm^3) in children with severe malaria against children with uncomplicated malaria (262.04 ± 157.13 mm^3). The levels of serum ferritin were comparable in children with severe malaria (394 ng/ml range 35–2,500) and children with uncomplicated malaria (441 ng/ml; range 28–2,500) (Table 1). The level of CHIT activity in children with severe malaria (median 138 nmol/ml/h; range 13–521) was also comparable to that in children with uncomplicated malaria (median 151 nmol/ml/h; range 13–491) and the difference between the two groups was not significant. In children affected by malaria and in healthy controls, we did not encounter subjects with very low (< 2 nmol/ml/h) or undetectable plasma CHIT activity.

The correlation between Hb levels, platelet count and CHIT activity both in children with severe and uncomplicated malaria was not significant, A significant correlation ($r^2 = 0.47$, $p < 0.01$) between plasma CHIT activity and serum ferritin was found only in children with uncomplicated malaria (Fig. 1), while this correlation was not significant ($r^2 = 0.04$) in children with severe malaria (Fig. 2).

Table 1. Haematological data of 62 children with acute *P. falciparum* malaria, according to the clinical severity and healthy controls

Parameters	Severe (n = 22) (A)	Uncomplicated (n = 40) (B)	Healthy controls (n = 140) (C)
Age (months)	12.5 (2–70)	23 (9–140)	22 (10–100)
Leukocyte × 10^3 (mm^3)	17.5±12.6*	17.1±6.97†	8.53±2.74
Red cells × 10^6 (mm^3)	1.40±0.61°*	3.10±0.70†	3.95±0.78
Haemoglobin (g/dl)	3.75±1.26°*	8.30±1.58†	9.6±2.28
Ht (%)	11.5±3.8°*	24.6±5.01	26.4±4.38
MCV (u3)	84.5±18.8$	73.8±13.4†	81.55±11.48
MCH (YY)	27.6±5.94§	25.4±4.43	24.52±4.40
MCHC (%)	32.40±4.41£	33.38±3.48†	30.21±2.66
Platelets (mm^3)	141.85±61.96°*	262.04±157.13?	324.12±102.25
Ferritin (ng/ml)	394 (35–2500)*	441 (28–2500)†	120 (15–140)
Plasma chitotriosidase activity (nmol/ml/h)	151 (13–491)*	151 (13–521)†	72 (14–150)

A?--> B °$p < 0.0001$, $p = 0.012$; A --> C *$p < 0.0001$, § $p = 0.004$, £ $p = 0.001$; B --> C †$p < 0.0001$, ?$p = 0.003$. Ht— Hematocrit; MCV— Mean cell volume; MCH— Mean corpuscular haemoglobin; MCHC— Mean corpuscular haemoglobin concentration.

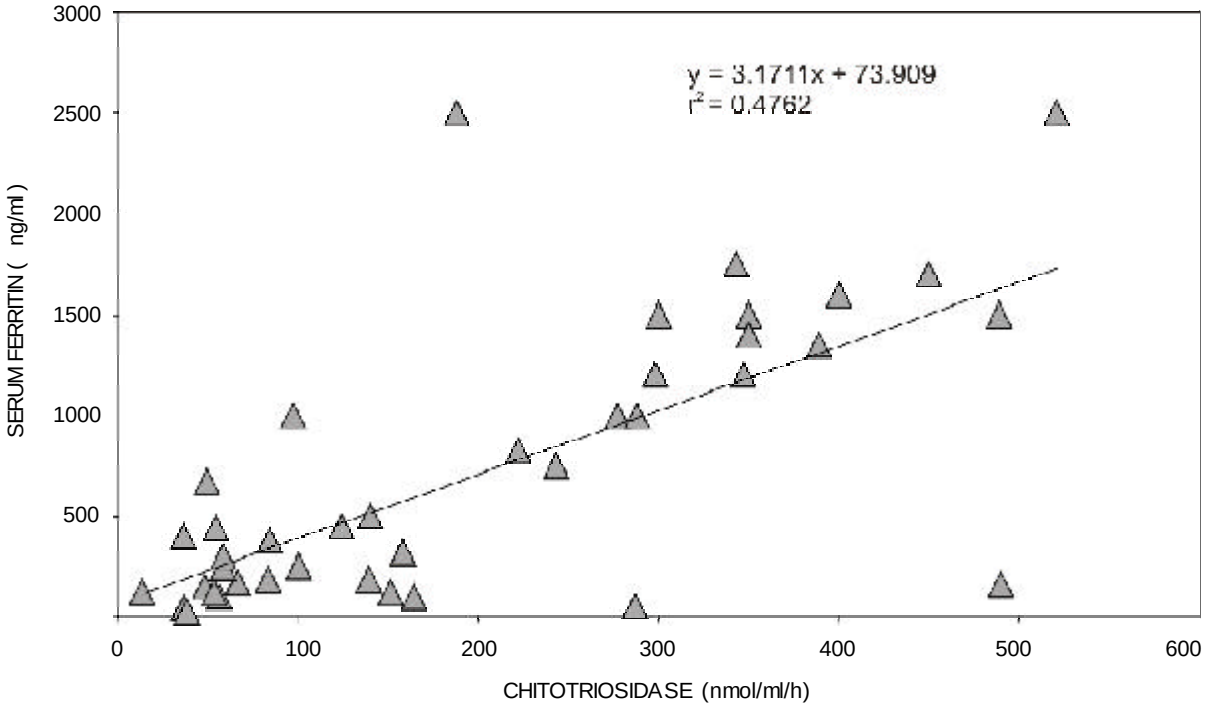

Fig. 1: Correlation between plasma chitotriosidase activity and serum ferritin in 40 children with uncomplicated malaria

In humans, CHIT is synthesised by activated macrophages and plasma CHIT activity has been proposed as a surrogate marker of macrophage activation[1]. In this study both children with severe and uncomplicated malaria had increased CHIT activity with respect to that in healthy controls, but the correlation between plasma CHIT activity and serum ferritin was found only in children with uncomplicated malaria. This observation suggests that in the immune response to *P. falciparum* the function of the CHIT gene plays an important role in the cascade of events in which the macrophage activation provides a crucial defence mechanisms against malaria[4]. In fact the difference between uncomplicated and severe malaria suggests two patterns of macrophage response: (i) a group with uncomplicated malaria where the plasma CHIT activity and serum ferritin levels correlated positively (Fig. 1); and (ii) a group with severe malaria, where the CHIT levels did not synchronize with the increase of serum ferritin (Fig. 2).

In such conditions the outcome of malaria infection and of other parasitic diseases could be dependent on the levels of plasma CHIT activity, but the lower CHIT activity observed in 7/22 (31.8%) and in 14/40 (35%) children with uncomplicated and severe malaria respectively (Figs. 1 and 2) can not be considered a consequence of a defective CHIT allele, since in Burkina Faso the percentage of heterozygotes for this allele is only 1–2%[5]. Hence, from these data we can rule out a genetic factor for the diversity in plasma CHIT activity, even if the CHIT activity may be related to previous exposure to parasitic infection so that the confounding effect of age in the results presented can not be excluded.

It is universally agreed that *P. falciparum* infection is mediated by the immune system and that a synchronic macrophage response facilitates the favourable outcome of infection with this parasite[4], but we do not know what mechanism regulates the expression of CHIT gene. The positive correlation

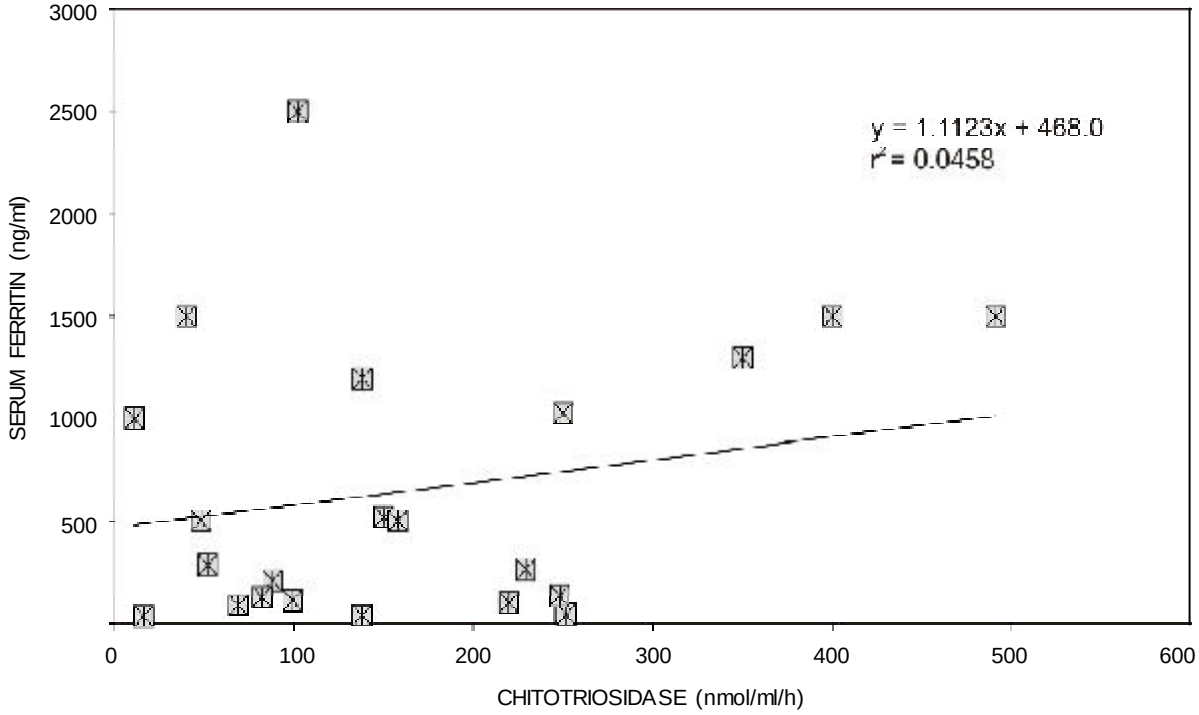

Fig. 2: Correlation between plasma chitotriosidase activity and serum ferritin in 22 children with severe malaria

between plasma CHIT activity and serum ferritin only in children with uncomplicated *P. falciparum* malaria, suggests that in severe malaria a failure of the " synchronic macrophage response", which may be an important determinant of disease outcome.

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