African Journal of Biotechnology Vol. 10(33), pp. 6319-6324, 6 July, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.043
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Predictors of childhood severe malaria in a densely populated area: Douala, Cameroon

Dongho Dongmo F. F.[1], Ngono Ngane R. A.[1]*, Gouado I.[1], Pankoui Mfonkeu J. B.[1], Mbackop Kwemba V.[1], Ngwa V.[2], Fotso Kuate H.[3] and Amvam Zollo P. H.[4]

[1]Department of Biochemistry, University of Douala, P. O. Box 24157 Douala, Cameroon.
[2]Paediatric Service, Laquintinie Hospital, Douala, P. O. Box 106 Douala, Cameroon.
[3]Laboratory Service, Laquintinie Hospital, Douala, P. O. Box 106 Douala, Cameroon.
[4]Department of Biochemistry, University of Yaoundé I, P. O. Box 812 Yaoundé, Cameroon.

Accepted 20 May, 2011

The physiopathology of malaria is complex. More understanding would be useful for a better management of the disease. This study was undertaken to describe clinical presentation and some biochemical parameters in childhood malaria in order to identify some factors of disease severity. Eighty six (86) children (0 to 15 years old) were recruited in Douala, clinical data recorded and blood sample collected. Thirty one (31) healthy children were also targeted to serve as control. Blood glucose, hemoglobin, transaminases and nitric oxide were determined by spectrophotometry. C reactive protein (CRP) was also investigated. The results confirmed that severe malaria significantly affects children under 5 years. Severe malaria was associated with hyperpyrexia and prostration. Coma, convulsions and unconsciousness were more indicative of cerebral malaria. Hemoglobin and blood glucose levels decreased significantly in severe malaria patients compared with uncomplicated malaria patients or controls ($P < 0.001$). On the contrary, blood transaminases and CRP levels increased significantly in malaria patients compared to controls ($P < 0.001$). From these results, it is clear that childhood severe malaria is associated with prostration, coma, unconsciousness, convulsions and hyperpyrexia. Low levels of haemoglobin and glycemia, as well as high levels of transaminases and CRP has been identified as predictor of malaria severity.

Keywords: Childhood malaria, clinical presentation, physiopathology.

INTRODUCTION

Malaria is still a major public health concern worldwide (Oshnishi, 2009). It affects about 300 to 400 million people and causes 1 to 2 million deaths each year, mostly African children (WHO, 2010). Eighty percent of these deaths occur during the first 24 h following admission (Dzeing-Ella et al., 2005). Even though many studies have been done on the physiopathology and management of malaria in different areas of Africa (Pankoui et al., 2008; Gouado et al., 2007; Dzeing-Ella et al., 2005; Severov et al., 2000; Kouamé et al., 2002; Tchokonteu et al., 1999), mortality remains high, particularly in children under five years of age, pregnant women and international travelers with no immunity against malaria. Severe infection to *Plasmodium falciparum* however, decreases after 5 years in the endemic areas (Genton and Acremont, 2000).

Malaria anemia (MA) and cerebral malaria (CM) are considered to be major manifestations of severe childhood malaria. Their pathogenesis is multi-factorial and not fully understood; there is then the need to

*Corresponding author. E-mail: angono@yahoo.com. Tel: + 237 77 81 16 35.

Abbreviations: MA, Malaria anaemia; **CM,** cerebral malaria; **UMC,** uncomplicated malaria; **CRP,** C reactive protein

address their complex etiology (Pankoui et al., 2008, Mishra et al., 2006). Most often, severe malaria is diagnosed lately and consequently, management is inefficient (Idro et al., 2005). Also, the prevalence of various malaria complications extremely differs with the setting, seasons and is very dependant of the genetic variations of the population involved (Labie and Tamouza, 2004). More investigations for a better understanding of childhood severe malaria would lead to an early diagnosis and therefore adequate and effective management.

The objective of this study was to describe clinical features and study some biochemical markers of childhood malaria in order to improve our understanding of the disease severity. The aim was to understand the association of those markers with malaria morbidity in our area and particularly to investigate their variation according to the different forms of malaria: Uncomplicated malaria (UCM), MA and CM.

MATERIALS AND METHODS

Study area

This study took place from July to October 2009 in the pediatric wards of 2 hospitals in Douala: Laquintinie and Deido District hospitals. Douala is the most popular town of Cameroon (14.4% of the Cameroonian's population, that is, 2,510,283 inhabitants) (I. Essono, 2010, statistics on Cameroon population in 2010, *CRTV News of 15/04/2010*), situated near the Atlantic Ocean (between 4 and 5° north and 9 to 10° east) where malaria is endemic all year round and is a major cause of morbidity and mortality. The protocol of this study was reviewed and approved by the Cameroon Bioethic committee.

Definition of groups

Anemia was taken as a hemoglobin concentration of <8 g/dl. MA was defined as a hemoglobin concentration of this level in a patient who had a positive thick film. CM was diagnosed if a patient with positive thick film presented with impaired consciousness as measured by a Blantyre coma score ≤2 (range: 0 to 5) and had normal cerebro-spinal fluid. Children without any of the mentioned symptoms, but presenting with usual malaria symptoms and a positive thick film were classified as UCM patients.

Study population

All children 15 years of age and under, who presented in participating hospitals were screened for the study. Children with sickle cells anemia, diarrhea and non-malaria infections (HIV infection and typhoid for instance) were excluded. Finally, after informed consent, eighty six (86) subjects with different forms of malaria were recruited. Thirty-one (31) children coming to the hospital for vaccination or nutritional counseling were recruited as controls, provided they were malaria free after thick films examination. 5 ml of blood was taken by venous puncture. Also, a drop of blood was used for malaria diagnosis. Subjects were assigned to different groups following WHO 2000 criteria for severe malaria (Saissy et al., 2003).

Baseline evaluation

Anthropometric data, information on the use of impregnated bed net to prevent malaria (whether or not net was used to cover the bed when resting or sleeping) or on recent drugs used and significant medical history were obtained. A complete physical examination, including neurological status according to the Blantyre coma score (Molyneux et al., 1989) and prostration assessment (defined as the inability to sit unassisted in a child who can normally do so or the inability to drink in a child who cannot normally sit up) were performed.

Malaria diagnosis

Blood was spotted on the slide for the preparation of the thick films. The films were dried, stained using GIEMSA stain and then examined with a microscope (Unico®, Dayton, USA). The result was expressed as the number of parasites per 100 leukocytes; this was converted to the number of parasites per microlitre of blood, taking into account the leucocyte count of the subject.

Biochemical markers determination

Hemoglobin as well as blood levels of glucose, transaminases (GOT, GPT) and nitric oxide were assayed by colorimetric method (using a spectrophotometer: Helios β Thermospectronic UVB 102615, Cambridge, United Kingdom). Hemoglobin was determined by the Drabkin method (*QCA:* hemoglobin-cyanoheamoglobin method, Amposta, Spain*).* Glycemia was done by enzymatic method using glucose oxidase and peroxidase (SGMitali: Glucose LR, Rome, Italia). Transaminases were assayed following the oxidation of NADH to NAD^+ in a series of reactions (Fluitest: GOT-ASAT, Lichtenfeis, Germany; Biorex: ALAT-GPT, Antrim, United Kingdom). Nitric oxide was determined following the conversion of nitrates in nitrites by nitrate reductase (R and D Systems, Minneapolis, *USA*). C reactive protein was determined by rapid agglutination test for qualitative and semi-quantitative detection (*Fortress diagnostics, Antrim, United Kingdom*).

Statistical analysis

Data were analyzed using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com. Kruskal-Wallis test was used to compare the median of all groups. Dunn multiple comparison test was used to compare the means between different groups. Relationships between the parameters were done using Pearson correlation test. P-values were used as measure of significance. $P < 0.05$ was considered significant.

RESULTS

Figure 1 is an overview of the patient's recruitments profile. From the 181 subjects screened, 117 were eligible and were finally enrolled after informed consent. Of those 117 recruited subjects, we had 31 controls and

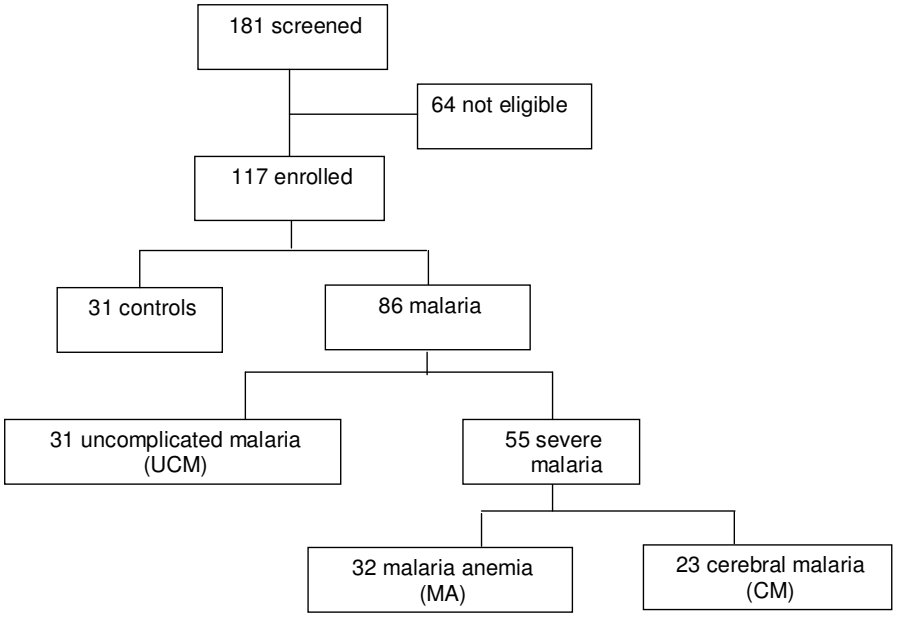

Figure 1. Study profile of patient.

Table 1. Baseline characteristics of malarial patients according to disease severity and controls on admission.

Parameter	Group				P
	Control (n= 31)	UCM (n= 31)	MA (n= 32)	CM (n= 23)	
Patients characteristics					
Age (months)	40.13±33.13[a]	30.97±29.64	38.94±31.85	37.69±31.83	ns
Male n (%)	17 (54.8)	16 (51.6)	14 (43.25)	11 (47.8)	NR
Weight (kg)	15.47±6.64[a]	13.02±7.76	14.21±6.17	14.26±6.16	ns
Temperature (°C)	37.10±0.28[a (≠)]	38.23±0.77[(#)]	38.39±1.16[(#)]	39.00±1.01[(‡)]	***
Laboratory data					
Parasitemia (Parasites/µl)	--	1200[b(≠)]	13500[(#)]	24000[(#)]	***
Hemoglobin (g/dl)	15.28±4.21[a (≠)]	12.39±2.22[(≠)]	6.27±1.76[(#)]	8.71±3.47[(#)]	***
Glycemia (g/l)	0.83±0.16[a (≠)]	0.68±0.21[(#)]	0.69±0.21[(#)]	0.65±0.14[(#)]	**
GOT (U/l)	37.35±15.82[a (≠)]	57.72±31.47[(#)]	56.93±30.48[(#)]	54.19±19.12[(#)]	***
GPT (U/l)	28.25±13.42[a (≠)]	46.11±30.29[(#)]	49.04±29.48 [(#)]	43.46±13.89[(#)]	***
CRP (mg/l)	(0.0)[c(≠)]	(0.0-24)[(#)]	(1.50-48.0) [(#)]	(15.0-70.0)[(#)]	***
NO (µmol/l)	50.81±21.28[a]	52.48±15.31	55.28±20.14	61.93±24.10	ns

a: Means±SE; b: geometric means; c, interquartiles, GOT, glutamate oxaloacetate transaminases; GPT, glutamate pyruvate transaminases; NO, nitric oxide; CRP, C reactive protein; MA, malaria anemia; CM, cerebral malaria; UCM; uncomplicated malaria; P, probability, ns, not significant at 5%, **: significant at 1%, **: significant at 0.1%, NR: not relevant. Values affected with the same sign (≠), (‡) or (#) are not significantly different at 5%.

86 malaria subjects including 31 with UCM and 55 with severe malaria (32 MA and 23 CM). Table 1 shows the baseline characteristics of controls and malaria patients. Table 2 presents the prevalence of some abnormalities and use of bed net. It appears that children under 5 years were significantly affected by severe malaria (81.25 and 87% for respective groups MA and CM) and had a low frequency of impregnated bed nets utilization (58.1, 45.2, 28.1 and 23.7% prevalence of bed utilization in controls, UCM, MA and CM, respectively). The majority of patients were living in populous, dirty and swampy areas of the town (Oyack, Village, Ndog-bong, Bepanda, Dakar,

Table 2. Prevalence of some abnormalities and use of bed net according to disease severity on admission.

Abnormalities and used of bed net (%)	Group			
	Control (n= 31)	UCM (n= 31)	MA (n= 32)	CM (n= 23)
Children under 60 months	21 (67.7)[a]	20 (64.5)	26 (81.25)	20 (87.0)
Use of bed nets	18 (58.1)[a]	14 (45.2)	9 (28.1)	5 (23.7)
Hyperpyrexia (T≥40°C)	0 (0.0)[a]	1 (3.2)	3 (9.4)	7 (30.4)
Prostration	0 (0.0)[a]	7 (22.6)	23 (71.9)	23 (100)
Coma	0 (0.0)[a]	0 (0.0)	0 (0.0)	19 (82.6)
Impaired consciousness	0 (0.0)[a]	0 (0.0)	0 (0.0)	23 (100)
Convulsions seizures	0 (0.0)[a]	0 (0.0)	1 (3.1)	21 (91.3)
Hyperparasitemia (>250000 Pa/µl)	-	0(0.0)	2(6.2)	7(30.5)
Low hemoglobin (<11 g/dl)	2(6.4)[a]	12(38.7)	32(100)	16(69.9)
Hypoglycemia (<0.7 g/l)	2(6.4)	15(48.8)	16(50.0)	14(69.9)
High GOT (≥41 U/l)	7(22.6)[a]	19(61.3)	19(59.4)	16(69.6)
High GPT (≥31 U/l)	7(22.9)[a]	18(58.0)	20(62.5)	17(73.9)
High CRP (>6 mg/l)	7(22.6)[a]	21(67.7)	24(75.0)	21(87.0)
High NO (> 97 µmol/l)	0(0.0)[a]	0(0.0)	4(12.5)	4(17.4)

a: Number (percentage), Pa: parasites, GOT; glutamate oxaloacetate transaminase; GPT, glutamate pyruvate transaminase; NO, nitric oxide; CRP, C reactive protein; MA, malaria anemia; CM, cerebral malaria; UCM, uncomplicated malaria.

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Clinical data on admission

According to Table 1, temperature on admission was significantly higher in CM patients than MA and UCM patients (39±1.01°C, 38.39±1.16°C, 38.23±0.77°C, respectively; P < 0.0001). Moreover, from Table 2, it appears that hyperpyrexia (temperature ≥ 40°C) was more prevalent in CM patients (30.4%) than MA patients (9.2%) and UCM (3.2%). All the children with CM were prostrated on admission while we had 71.9 and 22.6%, respectively with MA and UCM patients. Also, all CM patients had an altered conscience on admission and the majority of them were in coma (82.6%) and had fit (91.3%) on admission, a situation not observed in MA and UCM patients.

Laboratory data

From Table 2, we noted that 6.2% of MA patients and 30.7% of CM patients had hyperparasitemia (>250000 Parasites/µl) while none in UCM group was hyperparasitemic. In fact, Table 1 shows that the average parasitemia significantly increases from UCM patients to CM patients (1200, 13500 and 24000 Parasites/µl respectively in UCM, MA and CM patients, p < 0.0001).

Low hemoglobin level (<11 g/dl) was significantly prevalent in severe malaria patients (100% of MA patients and 69.6% of CM patients) compared with UCM patients (38.2%) and controls (6.2%). In fact, the average hemoglobin level significantly decreases in severe malaria patients compared to UCM patients and controls (15.28±4.21, 12.39±2.22, 6.27±1.76 and 8.71±3.47 g/dl, respectively or controls, UCM, MA and CM; P < 0.0001). Moreover, negative and significant correlation was observed between hemoglobin and parasitemia on the malaria patients (r= -0.2789; P= 0.0147).

Prevalence of hypoglycemia was significantly higher in malaria patients than controls and increased with severity of disease (6.2, 48.8, 50 and 60.9%, respectively to controls, UCM, MA and CM). Glycemia was significantly lower in CM patients than in controls (0.83±0.16, 0.68±0.21, 0.69±0.201 and 0.65±0.14 g/l respectively for controls, UCM, MA and CM; P= 0.0028).

Furthermore, prevalence of high levels of GOT was 61.3, 59.4 and 69.6%, respectively in children with UCM, MA and CM, while prevalence for high GPT was 58, 62.5 and 73.9%, respectively in children with UCM, MA and CM. High levels of GOT and GPT were observed on 22.6 and 22.9% of controls patients, respectively. Consequently, transaminases levels were significantly higher in malaria patients than controls. For GOT, the mean levels obtained were 37.35±15.82, 57.72±31.47, 56.93±30.48 and 54.19±19.12 U/l, respectively to controls, UCM, MA and CM patients (P= 0.0006). For GPT, the mean levels obtained were 28.25±13.42, 46.11±30.29, 49.04±29.48 and 43.46±13.89 U/l, respectively to controls, UCM, MA and CM patients (P= 0.0003). We also noted a significant and positive correlation

between the levels of the two transaminases (r = 0.5091, P < 0.0001). However, no significant correlations were observed between parasitemia and transaminases.

It was also noteworthy that 67.7, 75.0 and 87.0% of children with UCM, MA and CM, respectively had high levels of CRP, for only 22.6% in control subjects. Children with severe malaria had very high CRP level (>96 mg/l) compared with those with UCM (0.0, 18.7 and 26.1% in children UCM, MA and CM).

High NO levels (>97 µmol/) were observed only in children with severe malaria (12.5 and 17.4% in MA and CM, respectively). However, the majority of malaria patients (67.7, 62.5 and 60.8% in UCM, MA and CM patients, respectively) had NO levels between 50 and 97 µmol/l for only 45.2% in controls. Furthermore, NO levels increased in malarial patients according to the severity of disease (50.81±21.28, 52.48±15.31 and 55.28±20.14 and 61.93±24.10 µmol/l in controls, UCM, MA and CM patients, respectively). Therefore, significant correlation was observed between NO levels and some parameters particularly temperature (r= 0.6733, P < 0.0001), parasitemia (r= 0.3176, P= 0.0052) and CRP levels (r= 0.4722, P= 0.0472).

DISCUSSION

This study on childhood malaria was designed to describe the clinical spectrum and some biochemical parameters, as well as to understand the trend in pattern of morbidity of disease in our setting, particularly to look for variation in different disease severity groups. We found that children under 5 years, irrespective of the sex, were significantly affected by severe malaria. The high rate of infection in young age patients could be due to low immunity of children under 5 years (Genton and D'Acremont, 2000) and early abandon of chimio-prophylaxis according to WHO recommendations. We also found that there seems to be a strong link between bed net usage and the reduction of the disease severity. This proves that impregnated mosquitoes bed net to prevent malaria transmission is still working and remains a very interesting tool to implement in order to prevent severity when it occurs (Noor et al., 2007). Moreover, we noticed that the majority of patients were living in populous areas of the town. In fact, these areas were most of the time dirty and/or swampy, offering fitting breeding sites for the malaria vector.

Prostration, coma, unconsciousness and convulsions, significantly occurred in children with severe malaria, although, coma and convulsions seem to be specific to CM. This confirms their appropriateness in the WHO (2000) criteria of severe malaria identification (Saissy et al., 2003).

Parasitemia was significantly higher in children with severe malaria. Previous studies showed positive correlation between parasitemia and severity of malaria (Pankoui et al., 2008; Tchokoteu et al., 1999). In fact, organs and/or metabolic dysfunction which characterize severe malaria are due to the presence of *P. falciparum* in organ deep microvessels (Saissy et al., 2003).

Significant decrease of hemoglobin in severe malaria patients would be explained by the interaction of several factors (Ladhani et al., 2007) particularly the massive destruction of infected red blood cells during the schizogony; the phagocytosis of infected and non infected red blood cells and the erythropoiesis dysfunction due to increase in levels of TH1 type cytokines which affects marrow bone function. These hypotheses are corroborated by the significant and negative correlation (r= -0.2789, P= 0.0147) between parasitemia and hemoglobin in patients.

Hypoglycemia noted in malaria patients is an important complication of *P. falciparum* malaria and could be due to a failure of hepatic neoglycogenesis, but also to an overconsumption of glucose by parasite (Mishra et al., 2006; Saissy et al., 2003).

Concerning the two transaminases (GOT and GPT), their activity was significantly high in malaria patients. The strong significant correlation (r= 0.5091, P < 0.0001) between their values confirms these observations. These results corroborate with those obtained by Bourée et al. (2000) who noted an increase of transaminases in 80% of malarial subjects. Moreover, Shah et al. (2009) showed the increase of transaminases in association with malaria jaundice in patients. The increase of transaminases could be due to the fact that after infection, the *Plasmodium* causes the lysis of hepatocytes during its exo-erythrocytic cycle. This lysis leads to the liberation of hepatic enzymes like transaminases in blood. This process happens irrespective of the severity of malaria, that is, the form of the disease, whether UCM or severe malaria. This goes in line with the non significant correlation obtained between parasitemia and transaminases.

C reactive protein (CRP) increases with the severity of the disease, as noted in other studies (Nahrevanian et al., 2008; Bourée et al., 2002; Kremsner et al., 1997). In fact, in case of inflammation, CRP concentrates on affected tissues to exert its biological properties: complement activation, indirect bacterio-static effect facilitating an ingestion of microorganisms and resorption of injured tissues by phagocytosis and platelets aggregation activation. Besides, CRP will prevent the penetration of sporozoites in hepatocytes. Also, during malaria, mononuclear cells activated by *Plasmodium* produce inflammatory cytokines such as TNFα and IL-1 and 6. These cytokines stimulate hepatic synthesis of inflammatory reaction proteins as CRP, orosomucoide and heptoglobulin which are high during malaria (Nahrevanian et al., 2008; Bourée et al., 2002).

We equally found no significant increase of nitric oxide

levels in malaria patients and with severity of diseases. These observations were in line with several previous studies (Nahrevanian et al., 2008; Keller et al., 2004; Gyan et al., 2000). Labie and Toumaza (2004) show that during malaria, high NO levels will have not only a protective effect against diseases, but also toxic effect for *P. falciparum*. Moreover, increase of NO level during malaria will be a consequence of malaria severity. In fact, high level of NO production stimulators which are pro-inflammatory cytokines (TNFα and IL-10) is classically correlated with malaria severity. This affirmation goes in line with the significant and positive correlation between NO and some parameters as temperature ($r= 0.6733$, $P < 0.0001$) and CRP ($r= 0.4722$, $P = 0.0472$). Keller et al. (2004) showed an association between decrease of hemoglobinemia and increase of NO levels which is probably due to NO synthethase 2 (NOS2) induction by hemozoïn which is a hemoglobin derived compound from *P. falciparum* infecting erythrocytes. This increase of NO levels will be able to weaken the neuronal signalization and to damage the erythrocytes and thus, contributes to anemia complication and/or cerebral attack (Sobolewski et al., 2005). These results are confirmed by significant and positive correlation between NO levels and parasitemia ($r = 0.3176$, $P = 0.0052$).

Conclusion

This study highlights differences in clinical presentation as well as in some biochemical parameters according to disease severity. Most deaths from malaria occurred in the first 24 h of admission, which highlights the need for early recognition of the most severely ill children. Early diagnosis and classification of severe malaria would allow appropriate management. Thus, in addition to the basic clinical patterns as prostration, coma, convulsion and impaired consciousness, it will be interesting to take into account other parameters like hemoglobin and glycemia as well as levels of transaminases and CRP.

ACKNOWLEDGEMENTS

The authors thank the medical staff of participating hospitals. They are also very grateful to the study participants and their parents/guardians.

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