Original Article

Anaemia and severe malarial anaemia burden in febrile Gabonese children: a nine-year health facility based survey

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Abstract

Introduction: Anaemia remains a major cause of poor health in children and pregnant women living in sub-Saharan Africa. Malaria is one of the main causes of anaemia in endemic countries. At the time of decreasing *Plasmodium falciparum* infection prevalence among children, it was essential to analyze the evolution of anaemia and severe malarial anaemia (SMA), the most frequent clinical manifestation of severe malaria, in Gabon.

Methodology: Yearly recorded haemoglobin levels of febrile children aged below11 years, who benefitted from microscopic malaria diagnosis, were retrospectively analyzed to determine the evolution of anaemia and SMA prevalence throughout a nine-year period between 2000 and 2008.

Results: Anaemia prevalence remained high both in *P. falciparum*-infected children (between 87.6% and 90.7%) and in uninfected children (between 73.5% and 82.6%). Although the risk of developing severe anaemia ranged between 1.9 [0.9-3.8] in 2000 and 3.0 [1.3-6.5] in 2007, SMA prevalence did not significantly change during the study period, varying from 6.0% to 8.0%. From 2001, the frequency of SMA was comparable between children younger than five years of age and children older than five years of age.

Conclusions: The decreasing malaria prevalence previously observed in Gabon between 2000 and 2008 was not associated with a significant reduction of anaemia and SMA burden among children. Furthermore, other factors such as nutritional deficiencies, which may not be negligible, must be investigated in this vulnerable population

Key words: anaemia; malaria; children; SMA; Gabon

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Introduction

The major causes of malaria-related death are severe anaemia (SA) and cerebral malaria (CM) [1,2]. The anaemia burden is high among pregnant women and children in sub-Saharan Africa. Though causes of anaemia are multifactorial in tropical areas, they are frequently associated with and more pronounced in cases of *Plasmodium falciparum* infection [3-5]. Severe malarial anaemia (SMA) is the major clinical presentation of severe malaria (SM) in hyper- and holo-endemic areas; it was responsible for 30% of mortality in young children in Kenya [5-7]. In 2001 and 2002, before the introduction of new malaria control strategies in Gabon, more than 60% of children hospitalized with severe malaria presented with SMA [8,9]. Recently, a threefold reduction in the number of malaria cases was observed in Libreville and the surrounding areas. This decline was accompanied by a shift towards a higher susceptibility in children older than five years of age for both uncomplicated and complicated malaria. This shift suggests an epidemiological transition of malaria transmission in Gabon [10]. The peak age of children with CM increases as control interventions are successful in decreasing transmission intensity [11]. The relationships between age patterns and SMA, however, are not clear. As SMA prevalence is the main clinical manifestation of SM in many endemic areas, its frequency should be influenced by interventions; an impact on disease manifestations can also be observed, as reported in northeastern Tanzania [12]. Monitoring the disease burden and the age patterns of *P. falciparum* infection-associated syndromes can help not only to assess the impact of control strategies, but also to design age-specific interventions. Using available hematological data from 2000 to 2008, we analyzed the evolution of SMA prevalence, the most frequent clinical form of SM in Gabon, at a time when

the prevalence of *P. falciparum* infection was decreasing.

Methodology

Study site and population

This was a retrospective, observational, and analytical study. Data were collected between August 2000 and December 2008 in Libreville [10]. The population of Gabon is estimated to be 1,534,381 inhabitants. Libreville, the capital city, has close to 600,000 inhabitants. Malaria transmission level is high in the city, predominantly caused by *P. falciparum,* with an annual mean entomological inoculation rate estimated at 33.9 infected bites per person per year [13]. The Malaria Clinical and Operational Research Unit (MCORU) is a branch of the Department of Parasitology of the Medicine Faculty, located in the Centre Hospitalier de Libreville (CHL), the largest public hospital in the country. This unit works closely with the paediatric wards and the haematology lab.

A blood sample was collected from each febrile paediatric outpatient and inpatient aged up to 11 years. The samples were routinely screened for malaria diagnosis based on microscopic examination. The childrens' dates of birth, the parasite densities of those who had available haemoglobin and MCV measurements were recorded during a nine-year period (2000 through 2008) and retrospectively analyzed.

Malaria diagnosis

Thick blood films were done for each patient and blood slides were processed according to the Lambaréné procedure [14]. Slides were considered negative if no asexual blood stages of parasites and gametocytes were seen in 100 oil-immersion fields. Quality control of the blood smears was performed by a second microscopist who was unaware of the results of the first reading. In case of disagreement, slides were controlled by a third tester, and the average of the two closest parasitaemia was taken. The case definition of malaria was a febrile child with slide-confirmed parasites (PBS). Parasitaemia was expressed as the number of parasite/µL of blood and classified as low (less than 100,000 p/µL) and high (equal or more than 100,000 p/µL). Hyperparasitaemia was defined as a parasite density of ≥ 250,000 p/µL

Haematological measurements

Haemoglobin (Hb) was measured using a Coulter counter (SKTS, Beckman Coulter Corporation, Brea, USA). According to the World Health Organization classification, anaemia was defined as an Hb concentration below 11 g/dL and classified as severe (Hb < 5 g/dL) (severe anaemia, SA), moderate (5 ≥ Hb < 8 g/dL) and low (8 ≤ Hb < 11 g/dL). Severe malarial anaemia patients presented SA and a *P. falciparum*-positive blood smear.

Microcytosis, defined as a low mean corpuscular volume (MCV), was determined according to age: MCV < 70 fl for children between six months to two years of age, MCV < 75 fl for children between two to six years, and MCV < 77 fl for children between six and ten years. Children with both low Hb and low MCV hadmicrocytic anaemia.

Ethical consideration

The study was approved by the public health ministry of Gabon. There was no additional blood collected from the children for this study. Data from the patients with available blood smears and hemoglobin results were analyzed. The childrens' parents or guardians were informed about the study, and their oral consent was required prior to data collection and reporting.

Data analysis

Demographic, clinical, and laboratory data of patients were recorded on a standardized data entry form and entered into the Epi-info version 6.0 (CDC, Atlanta, USA) database. Median parasitaemia was estimated only in the group of patients with positive blood smears. Data were analyzed using Stata version 9.2 (Stata Corporation, College Station, USA). Variables were summarized as frequencies and median [25th through 75th percentiles]. Differences between groups were assessed using Chi square or Fisher's exact test for proportions, Student's *t*-test and analysis of variance (ANOVA) or Kruskal-Wallis test as appropriate for continuous variables (age, haemoglobin and MCV levels, parasite density). The Pearson Chi square test for trend was used to identify the linear trend between groups. A separate stepwise backward multivariable logistic regression analysis to assess the effect of age and *P. falciparum* infection on anaemia and SMA prevalence was achieved. Two-way interaction terms between variables were tested. Odds ratio with their 95% confidence interval for either SMA and/or age equal to or greater than five years compared with either severe non-malarial anaemia and/or children below five years old were calculated. In the second step, the SMA-specific model was analysed using the same variables. A p-value of less than 0.05 was considered significant.

Figure 1: Yearly distribution of anaemia cases

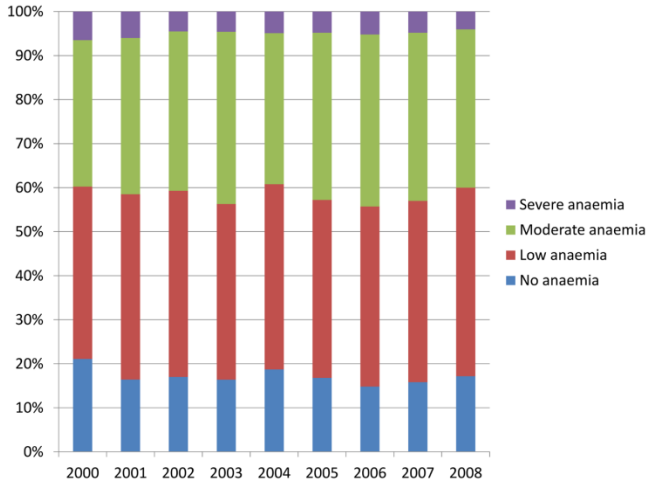
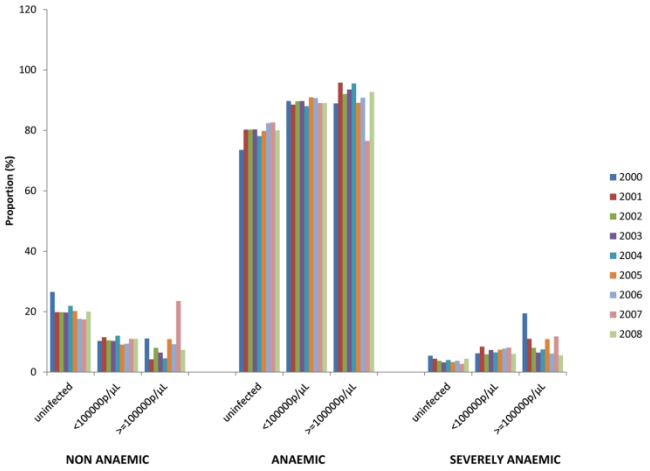


Figure 2: Yearly distribution of type of anaemia according to parasitaemia



Results

During the study period, hematological data were available for 16,383 patients. The total annual number of patients ranged from 417 to 3,807, with the highest number of patients recorded in 2002 and 2003. The median age was 24 months (Table 1). The difference of median ages observed between years was not statistically significant (p = 0.3) (Table 2a). Patients younger than five years old represented more than 70% of the febrile children.

Hematological characteristics of patients are presented in Table 1. The median Hb level remained below 9.7[7.9-10.6] g/dL during the study period. Throughout the years, the median Hb of malaria-infected patients was significantly lower (yearly values varying from 8.2 [6.7-10.3] g/dL to 8.7 [6.9-10.0] g/dL) compared to the uninfected patients (yearly values between 9.5 [8.5-10.5] g/dL and 9.8 [8.4-11.0] g/dL) (p < 0.01).

Type of anaemia

The proportion of non-anaemic patients varied from 21.1% to 14.1%. The rates of low and moderate anaemia were comparable between 2000 and 2008; the rates ranged from 39.2% to 42.4% for low anaemia and from 33.2% to 39.2% for moderate anaemia (Figure 1). A total of 818 children presented with severe anaemia. Global SA prevalence varied from 4.5% to 6.5% during the nine-year period (Figure 1, Table 2a).

Parasitaemia and type of anaemia

The proportion of anaemic patients remained significantly higher in case of PBS (87.6% to 90.7% versus 73.5% to 82.6% in malaria-free patients) (p < 0.01) (Table 2a). SMA rates (5.9%-8.7%) were always greater than non-malarial SA rates (2.6%-5.4%), in every year (p < 0.01). The risk of developing SA did not drastically change during the study period (p of trends = 0.2) (Table 2a). *P. falciparum*-infected patients were at significant risk of developing SA; this risk did not significantly vary during the study period (p of trends = 0.3) (Table 2a).

Parasite density was distributed as follows: from 2,000 p/µL to 12,000 p/µL in the non-anaemic patients; 7,000 p/µL to 14,500 p/µL in the low anaemic group; 8,144 p/µL to 15,436 p/µL in the moderately anaemic patients; and 4,460 p/µL to 18,000 p/µL in the severely anaemic group. Globally, anaemia and SA frequencies were the highest in the group of patients with high parasite density (> = 100,000 p/µL) (Figure 2). Hyperparasitaemia (> 250,000 p/µL) was detected in 3.2% (n = 182/5746) of children. This proportion remained below 4% throughout the study period (Figure 2). Among the *P. falciparum*-infected patients, the median Hb level was comparable between those with hyperparasitaemia (8.4 [6.9-9.6]g/dL) and those without (8.6 [6.9-10.0] g/dL) (p = 0.13).

Microcytic anaemia was more frequent among the malaria-free patients (48.9%) compared to the infected patients (60.8%) (p < 0.01).

SMA and age

The median age of patients varied from 18 [9-48] months to 24 [11-48] months of age; in SMA patients, it was between 24 [12-36] months, and 36[12-72] months in non-malaria patients with SA. The studied population was stratified into two groups, less than or equal to or greater than five years of age.

Table 1: Patients' characteristics

	All (n = 16383)		Infected (n = 5746)		Uninfected (n = 10637)		*P*
Age, months	24 [12-48]*		24 [12-36]		27 [12-60]		0.01
Parasitaemia, p/µL**			9500 [1500-49971]				
Haemoglobin,* g/dL	9.4 [7.8-10.5]		8.6 [6.9-9.9]		9.7 [8.4-10.7]		0.01
	n	%	n	%	n	%	
Anaemia	13656	83.4	5157	89.7	8499	79.9	0.01
Severe anaemia	818	5.0	411	7.1	407	3.8	0.01

*median [25th-75th percentiles]; **determined in the group of infected patients

Table 2: Distribution of severe anemia cases according to age and presence of *P. falciparum* infection

Table 2a

	2000	2001	2002	2003	2004	2005	2006	2007	2008
N	**634**	**2408**	**3807**	**3354**	**1805**	**1030**	**1331**	**417**	**1597**
Age, median	24[14-48]*	24 [12-48]	30 [14-60]	24 [12-48]	20 [13-60]	18 [9-72]	10 [10-60]	24 [12-60]	28 [24-60]
NON-MALARIA PATIENTS									
N	**404**	**1514**	**2543**	**2167**	**1172**	**687**	**828**	**244**	**1058**
Anaemic, n (%)	297 (73.5)	1241 (80.2)	2039 (80.2)	1741 (80.3)	914 (78.0)	548 (79.8)	682 (82.4)	218 (82.6)	846 (80.0)
SA, n (%)	22 (5.4)	66 (4.4)	95 (3.7)	70 (3.2)	46 (3.9)	23 (3.3)	31 (3.7)	7 (2.6)	45 (4.4)
***P. falciparum*-INFECTED PATIENTS**									
N	**230**	**894**	**1264**	**1187**	**633**	**343**	**503**	**153**	**539**
Anaemic, n (%)	206 (89.6)	800 (89.5)	1135 (89.8)	1071 (90.2)	562 (88.8)	311 (90.7)	456 (90.7)	131 (87.6)	482 (89.4)
SMA, n (%)	19 (8.3)	78 (8.7)	77 (6.1)	95 (7.2)	40 (6.6)	27 (7.9)	38 (7.6)	13 (8.5)	32 (5.9)
OR for SMA* **	1.9 [0.9-3.8]	1.7 [1.2-2.5]	1.6 [1.1-2.1]	2.0 [1.5-2.8]	2.2 [1.5-3.3]	2.5 [1.5-4.3]	2.3 [1.5-3.6]	3.0 [1.3-6.5]	1.4 [0.9-2.3]

*[25th-75th percentiles]; **Odds ratio for SMA risk with severe non-malarial anaemic patients as reference

Table 2b

	2000	2001	2002	2003	2004	2005	2006	2007	2008
Severe non-malarial anaemia									
[0-5 years]									
N*	291	1162	1838	1609	813	488	589	182	784
%**	5.8	4.3	3.6	3.4	3.9	3.1	3.3	3.3	3.5
[5-10years]									
N	113	352	705	558	359	199	239	82	274
%	4.4	4.5	3.9	2.7	4.2	4.0	5.1	1.2	6.3
Severe malarial anaemia									
[0-5 years]									
N	202	759	1023	958	502	268	403	117	433
%	8.9	9.2	5.7	6.8	6.4	7.4	7.9	10.3	6.0
[5-10 years]									
N	28	136	241	229	131	75	100	36	106
%	3.6	7.3	6.6	9.2	7.9	9.3	6.0	11.1	7.5
OR for SMA* **									
[0-5 years]	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
[5-10 years]	0.4 [0.1-3.11]	0.8 [0.4-1.6]	1.2 [0.7-2.1]	1.4 [0.8-2.3]	1.2 [0.6-2.5]	2.8 [1.2-6.9]	0.8 [0.3-1.9]	1.1 [0.3-3.6]	1.3 [0.5-2.9]

*total number; **proportion of children with Hb<5g/dL; ***: Odds ratio for SMA risk with [0-5 years] years SMA patients as reference

The proportion of patients aged less than five years with severe non-malarial anaemia remained below 4% after 2002. Except in 2005, the risk of having SA in the presence of *P. falciparum* infection was comparable between the two groups and did not significantly vary during the study period (χ^2 of trend = 13.1, p = 0.1) (Table 2b).

Multivariate analysis

Binomial logistic regression analysis showed that, taking into account the presence of malaria and age, the risk of developing anaemia did not differ from 2000 to 2008 in the *P. falciparum*-infected (aOR 1.1 [0.9-1.3], p = 0.4) and non-infected (aOR 0.9 [0.8-1.1], p = 0.4) children.

Discussion

Anaemia remains a major cause of poor health in children and pregnant women living in sub-Saharan Africa. Globally, up to 80% of African people are anaemic [15]. The present study highlights the permanently high burden of anaemia among children aged up to 11 years old, regardless of its association with malaria. The high rate of anaemic children with negative blood smears underlines the importance of other influencing factors such as nutritional deficiencies, HIV, hookworm, and urinary schistosomiasis that induce inflammatory anaemia. Their frequencies are below 15% in Gabonese children [16-18]. Sickle cell disease is found in only 4% of children. Alpha-thalassemia was described in a subgroup of children from an area with many migrants; it is less common than sickle cell disease [19]. Nutritional deficiencies, specifically iron deficiencies, are the first cause of anaemia in children in tropical areas. This should be the case for the majority of children consulting at the CHL who predominantly eat local food, which is enriched poorly with iron. Although less sensitive than hypochromia, microcytosis can be used for identification of iron deficiency anaemia [20]. Microcytic anaemia was more frequent among patients without malaria. Therefore, nutritional anaemia may preexist in early childhood and may be aggravated by other infections, symptomatic or asymptomatic, untreated or not, as is *P. falciparum* malaria. Indeed, in endemic areas, chronic anaemia is mostly due to iron deficiency, and incident cases, such as acute or non-tolerated moderate to severe anaemia, are due to malaria infection [21].

The number of children presenting with a fever varied dramatically from year to year. MCORU activities began in August 2000. Until 2004, the CHL was the largest pediatric consultation centre of the city and received the majority of febrile children. After 2004, several community health centers affordable for middle- and low-income populations were built in Libreville. The small number of screened children in 2007 was due to a strike of the paramedical staff that lasted more than six months at the CHL.

As reported in Malawi between 2001 and 2005, the evolution of the SMA frequency did not follow that of malaria prevalence at the same time in Libreville; the latter decreased from 45% in 2000 to 15% in 2008 in the paediatric wards of the CHL whereas anaemia, and SMA rates did not significantly change [10,22]. One explanation is that, whatever the malaria burden, the impact of *P. falciparum* infection on haemoglobin level remains the same. The frequency of other anaemia risk factors may have raised and enhanced the risk of SMA in children at the onset of malaria. The high risk of developing SMA in *P .falciparum*-infected patients confirms this hypothesis.

Identification of age groups bearing the highest burden of clinical symptoms due to malaria will enable better interventions. The decline in malaria transmission observed during the scaling up of new control tools was followed by a shift in the disease-attributable morbidity of older children [10,23,24]. Our previous reports showed a higher frequency of simple and complicated malaria in children older than five years old, when malaria prevalence decreased in the country [10,25]. It was therefore necessary to study the distribution of clinical syndromes due to malaria according to age. Indeed, the median age of patients with cerebral malaria (CM) increases with decreasing transmission intensity [26]. A similar trend was observed in the pediatric ward of the CHL where the median age of hospitalized children with CM was 18 months in 2002 and increased to 48 months in 2008 [8,25]. However, CM is still rare in Libreville, while SMA is the main manifestation of severe malaria (SM). Children aged below five years and those between 5 and 10 years were at similar risk of developing SMA in Libreville. The absence of a shift of SMA burden towards a specific age group confirms that SMA is not a good marker for the characterization of malaria transmission intensity in our settings, as reported by others [22]. However, the current risk of severe malaria syndromes in older children suggests a greater susceptibility to malaria in this group compared to 10 years ago [8]. It is not known whether this is due to a delayed acquisition of protective immunity, to a higher exposure, or to the uncontrolled

urbanization of the city that results in a hostile environment for mosquitoes. Indeed, *Anopheles gambiae sensu stricto*, the main vector of *P. falciparum,* represents only 12% of the mosquitoes in Libreville [13].

This study has two major limitations. Surveys at health facilities are not representative of the whole population. However, the present results approximate reality because almost half of the inhabitants of Gabon live in Libreville and the surrounding areas, and severe anaemia cases are usually transferred from peripheral health centers to the CHL for management. The second limitation is the lack of data on the history of the disease (*e.g.*, previous antimalarial self-medication). Recent reports suggest that 10% to 20% of febrile children had had previous antimalarial treatment at home; however, home treatment was not associated with a lower frequency of malaria [25]. The possibility that these patients would be more or less chronic carriers of *P. falciparum* and develop SA should be considered. This relationship would confirm the impact of inadequately treated infection on SMA, and also demonstrate the need to educate the population about the risks of self-medication. The proportion of febrile patients with negative blood smears who had had previous correct self-medication is often low; these patients would consult for another cause of fever.

Conclusion

This study is the first analysing the yearly evolution of anaemia and SMA prevalence in Gabon. The results highlight the high burden of anaemia and SMA among children. Malaria aggravates pre-existing anaemia. Despite the decrease of malaria prevalence observed in the city between 2000 and 2008, the impact of *P. falciparum* infection on SMA occurrence remained important and unchanged. Programs should improve the health of children of all ages through access to malaria control strategies, and by improving general nutritional status and life conditions.

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