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Hematological Profile of Malaria-Infected Patients from Bangui, Central African Republic: A Retrospective Study at a Diagnostic Medical Center

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Abstract

Background: Hematological disorders are the most common complications that determine the prognosis of malaria. Here, we compered hematological profiles of malaria-infected patients and malaria-negative patients at the Medical Analysis Laboratory of the Institute Pasteur in Bangui, Central African Republic. **Methods:** We conducted a retrospective study using archived data from 2 January to 30 December 2019 that included sociodemographic, parasitological, and hematological parameters. **Results:** The study showed that, out of the 518 subjects included, 28% were positive by microscopy. We detected a shift in the at-risk age group from the under 5 years age group to the 5–14 years age group. Approximately 47.49% of the study population and 69.5% of the children under 5 years were anemic. The mean values of hemoglobin were significantly lower in the malaria patients than the malaria-negative patients respectively 10.37 d/dL and 12.88 d/dL ($p < 0.0001$). The relative risk of anemia was higher (87%, RR = 1, 65%, OR = 5.96, $p < 0.001$) among malaria positive children, compared to malaria negative children. Neutrophil cell counts were significantly higher in the malaria-positive cases than in the controls. There was no significant difference in parasite density according to hematological parameters. **Conclusion**: Study participants infected with malaria demonstrated vital changes in hematological parameters, with anemia and neutrophilic leukocytosis being the most important associations observed in a retrospective laboratory-based study. Hematological alterations associated with malaria infection may vary depending level of malaria endemicity, back-

ground hemoglobinopathy, demographic factors and malaria immunity.

Keywords

Malaria, Hematological Parameters, Bangui, Central African Republic

1. Introduction

Malaria is the most prevalent parasitic infectious disease in humans and continues to be a major cause of morbidity and mortality worldwide and especially in Sub-Saharan Africa [1]. In the Central African Republic (CAR), malaria transmission continues to be a major public health concern, despite strengthened control interventions, with the whole population being at risk (5 million). An incidence rate of 310.6 cases per 1,000 population positions the Central African Republic as the seventh most affected country worldwide following Benin, Burkina Faso, Mali, Liberia, and Mozambique and the most affected country in Central Africa [2]. Children and pregnant women are the most vulnerable category of malaria patients, with malaria being the leading cause of death in children under 5 years old [3]. It has recently been shown that the prevalence of malaria in the CAR differs significantly among age groups, with high prevalence among children 1–4 years old, and spatial heterogeneity with higher prevalence in rural areas than urban areas [4]. An overview of malaria research in the CAR from 1987 to 2020 has shown that the leading research topics were drug evaluation, expatriate patients and malaria in children [5]. Importantly, few data have been published to date on the clinical pathophysiology and prognosis of malaria in the CAR.

The plasmodium parasite is a blood parasite and, during infection, the host-parasite interactions cause hematological alterations such as anemia, thrombocytopenia, leukopenia, and lymphocytosis and, infrequently, disseminated intravascular coagulation [6]. Although the parasite infects both hepatocytes and erythrocytes in humans, the symptomatology of the disease is only associated with the infestation of red blood cells. Malaria infection destroys erythrocytes, resulting in low hemoglobin levels known as anemia. The asexual blood stage of the parasite that infects the mature red blood cell (RBC) causes all of the symptoms and malaria-associated pathologies [7]. There is vast documentation demonstrating that the hematological consequences of malaria determine the clinical forms, severity and prognosis of the disease [8]. Malaria is commonly associated with alteration in the hematological cells of infected individuals in severe and acute uncomplicated phases [9]. Anemia due to malaria is indicated by the destruction of both infected and uninfected RBCs, a reduction in erythrocyte precursors and inhibition of erythropoiesis, eventually leading to severe malaria or death in patients [10].

In a study carried out in Bangui, the capital of the CAR, in 2010 in children, anemia and neurological manifestations were the major syndromes observed in severe malaria [11]. Severe malaria and its various clinical forms remain a major

problem for severe pediatric malaria in the CAR; understanding the hematological profile can help improve disease management [12]. Hematological alterations associated with malaria infection may vary depending on the following factors: demographic factors, level of malaria endemicity, hemoglobinopathy and malaria premunition [9]. However, there has been little research in the CAR investigating the effects of malaria on the hematological profile. Here, we assessed the hematological profile associated with malaria infection in Bangui patients. Therefore, the present study compared hematological indices of RBCs, WBCs and platelets between malaria-infected patients attending at the Diagnostic Research Center of Bangui and malaria-negative subjects.

2. Methods

2.1. Type and Site of the Study

A retrospective study of data from medical records of CAR patients with clinically suspected malaria at the Medical Analysis Laboratory at the Institute Pasteur of Bangui from January to December 2019. Inclusion criteria were based on complete availability of subject data. This laboratory, located near the center of the city of Bangui, receives middle- to high-income patients from all districts of the city.

2.2. Study Design

The data retrieved included subject demographics. Malaria diagnosis was done using a thick smears microscopy to determine the density of the malaria parasite. Thick and thin blood films were stained with Giemsa and microscopically examined for malaria parasites according to well-established guidelines [13]. Blood counts were performed using a Pentra ABX XRL Hematology Analyzer (Horiba) to determine the hematological parameters. Reference counts and cut-off values were used to interpret the results. The WHO defines anemia in children aged under 5 years as a hemoglobin (Hb) concentration of <11 g/dL and Hb levels of <12 g/dL in both sexes and for the population at large [14] [15]. Parasitaemia was classified into three categories according to the number of asexual stages/µl of blood: low (<1000), moderate (1000 - 9999) and high (≥ 10,000) [16] [17]. The inclusion criteria include patients for whom sociodemographic, parasitological and hematological data were available. Giemsa-stained blood smear microscopy was used to classify malaria status for subsequent hematological comparisons.

2.3. Data Analysis

Data were compiled using Epi-Info version 7.2.2.6. The statistical analyses were carried out using Stata software (version 14; Stata Corp, College Station, Texas, USA). The chi-squared test was used to assess differences in proportion and analysis of variance (ANOVA) test (depending upon the variable groups) to determine the relationship between malaria and sociodemographic characteristics as well as malaria and hematological parameters. Results were considered statistically significant when the p-value was < 0.05 at 95% level of significance.

3. Results

3.1. Sociodemographic Characteristics of the Study Participants

A total of 653 records were screened. Among these, 135 records were excluded due to incomplete data and duplicate visits from the same patients. This study included 518 subjects who met the inclusion and exclusion criteria, with 256 males (49.42%) and 262 females (50.58%), giving a sex ratio of 1:1.02. The average age was 22.8 years (SD = 18.5 years) (range: 0 months to 87 years). The 15 - 49 years age group was the most represented (48.07%), followed by 5 - 14-year-olds (20.46%) and 1 - 4-year-olds (15.83%).

3.2. Distribution of Malaria Infection According to Sociodemographic Characteristics

A total of 518 subjects were included in this study. Following the diagnosis results with Giemsa-stained thick blood film microscopy, we obtained 145 positive malaria cases corresponding a positivity rate of 28%. A statistical difference in prevalence was noted according to sex with 32% for males and 24% for females (p = 0.04). Furthermore, age groups showed highly significant differences: the 5 - 14 years age group was the most affected by malaria (58.49%), followed by the 1 - 4 years age group (47.5%) and the under 1 year age group (44%). For the 15 - 49 years and over 50 years age groups, prevalence was 11.24% and 4%, respectively. The risk of malaria infection was highest for the 5 - 14 years age group. There was no significant difference in parasite density by age, although the proportion of high parasitic density seemed to be more noticeable in the under 1 year age group and more than half of the subjects in that age group had moderate parasitic density (Table 1).

Table 1. Demographic characteristics of the study participants.

Variables	N	Malaria		p-value
		Positive n (%)	Negative n (%)	
Sexe				
M	256	82 (32)	174 (68)	0.04
F	262	63 (24)	199 (76)	
Age group				
<1 an	32	14 (44)	18 (56)	0.001
1 - 4 ans	82	39 (47.5)	43 (52.5)	
5 - 14 ans	106	62 (58.49)	44 (41.51)	
15 - 49 ans	249	28 (11.24)	221 (88.76)	
>= 50 ans	49	2 (4)	47 (96)	

3.3. Hematological Profile in *P. falciparum*-Infected Patients and Malaria-Negative Patients

The average hemoglobin concentration and the average mean corpuscular hemoglobin (MCH) were slightly lower in malaria-positive patients than in malaria-negative patients, with a statistically significant difference ($p = 0.0001$) for both parameters. In contrast, the neutrophil basophile and leukocyte counts were significantly higher in *P. falciparum*-infected patients than in malaria-negative subjects ($p = 0.003$ and $p = 0.02$, respectively). The average hematite, hematocrit, mean corpuscular volume (MCV) and eosinophil, lymphocyte and platelet counts were not significantly different between the two groups (Table 2). There was no significant difference in parasite density according to hematological parameters.

Table 2. Haematological profile of study participants.

Variables	Reference rang	Mean (SD)		*p-value*
		Malaria Positive	Malaria Negative	
RBC ($\times 10^6/\mu L$)	3.9 - 5.5	4.11 (2.9)	4.65 (0.04)	0.01
Hemoglobin (g/dL)	12 - 15	10.37 (0.21)	12.88 (0.33)	0.0001
Hematocrit (%)	37 - 47	31	45	0.29
MCV (fL)	80 - 100	79.62 (4.18)	80.71 (0.38)	0.68
MCH (pg/cell)	27 - 32	25.54 (0.25)	27.12 (0.15)	0.0001
MCHC (g/dl)	30 - 36	33.57 (0.25)	33.57 (0.06)	0.98
TLC ($10^3/\mu L$)	4 - 10	9.15 (0.35)	7.8 (0.4)	0.02
Neutrophils (%)	55% - 75%	69	48	0.003
Eosinophils (%)	1 - 5	0.7	0.9	0.17
Basophils (%)	<2	1.74	0.83	0.005
Lymphocytes (%)	20 - 45	29.14	33.3	0.53
Monocytes (%)	1 - 10	0.84	1.6	0.59
Platelet count ($\times 10^3$)	150 - 400	328 (4.9)	287 (1.2)	0.39

RBC: red blood cell, MCV: Mean Corpuscular Volume, MCH: Mean Cell Hemoglobin, MCHC: Mean Corpuscular Hemoglobin Concentration, TLC: Total leukocyte count.

The results demonstrated that more than half of the population study was anemic (47.49%, with Hb < 12 g/dL), and 52.51% had normal hemoglobin concentrations (Hb > 12 g/dL). The proportion of children under 5 years with anemia was 79/114 (69.5% with Hb < 11 g/dl). The relative risk of anemia was higher (87%, RR = 1, 65%, OR = 5.96, $p < 0.001$) among malaria positive children, compared to malaria negative children. It is of (75.8%, RR = 1.59, OR = 3.43, $p < 0.01$) among 5 - 14 years (Table 3).

Table 3. Prevalence of anemia according to the malaria status.

Age range	Hemoglobin level	Anemia prevalence (%)		*p-value*	RR	OR
		Malaria positive	Malaria negative			
Under 5 years	Hg < 11 g/dl	87% [81 - 93]	52% [44 - 60]	0.001	1.65	5.96
	Hg > 11 g/dl	13%	48%			
5 - 14 years	Hg < 12 g/dl	75.8% [69 - 83]	47.7% [39 - 55]	0.01	1.59	3.43
	Hg > 12 g/dl	24.2%	52.3%			
15 years and over	Hg < 12 g/dl	47% [39 -55]	21% [14 - 28]	0.01	2.19	3.24
	Hg > 12 g/dl	53%	79%			

4. Discussion

The prevalence of malaria observed in this study (28%) is lower than the national prevalence of 56% during the same period. The prevalence in Bangui was found to be relatively low compared with the rest of the country a pediatrics study showed a prevalence of 44% [4]. One reason for this relatively low prevalence is that the Medical Analysis Laboratory is frequented by middle- and high-income patients. Many studies have reported that malaria potentially affects the entire population in endemic areas; however, the poor and vulnerable are affected more mainly due to socio-economic factors. Furthermore, malaria is directly related to poverty and economic inequality on both the individual level and the national level [18]. Our study showed for the first time in the CAR a gradual shift in the at-risk-for-malaria age group from the under 5 years to the 5 - 14 years group. This result is similar to a study carried out in three villages in western Kenya showing higher prevalence of malaria among 5 - 14-year-old children [19]. This change in the age group at-risk or highly vulnerable indicates that the immunoimmaturity period has changed and that children under 15 acquire malaria premunity (gradually acquired, short-lived immunity). The clinical malaria vulnerability of this age group should be monitored for severe cases.

The causative agent of malaria is a hematophagous parasite, *Plasmodium* spp. and its pathophysiology modifies the hematological parameters that determine the prognosis of the disease. Thus, hematological abnormalities are considered a hallmark of malaria and are reported to be most pronounced in malaria due to *P. falciparum* infections [20] [21]. It has been demonstrated that, during a malarial infection, the parasite must obtain iron by lysing erythrocytes from its host to survive and replicate [22]. Our results showed that the hemoglobin level and the MCH in patients are significantly lower than in controls. Furthermore, our study revealed that more than half of the population study was anemic (47.49%), but 52.51% had normal hemoglobin concentrations despite infection with malaria. This result was higher in children under 5 years old, with 69.5% presenting anemia. Although anemia is highly prevalent among young children in high malaria

transmission settings, other factors such as malnutrition can be associated with anemia [23] [24]. Malnutrition and malaria are well-known causes of childhood anemia in tropical Africa and malnutrition is known to be a factor that influences hematological parameters [25]. A survey of nutritional status in the CAR shows that the prevalence of malnutrition is of 69.6% among children under 5 [26]. Although the medical literature has shown that the prevalence of anemia is highest in malaria-endemic areas, the main cause of anemia can nevertheless be traced to the diet (iron content), not malaria. In addition to malnutrition that can affect hematological parameters, sickle cell disease—a hemoglobinopathy—is a significant factor associated with hematological parameters, particularly in the CAR where this affliction affects 2% of infants, according to one study conducted in 2011 [27]. Whether in children or adults, infectious diseases such as HIV and hepatitis B, both caused by viruses with high prevalence rates in the CAR, or diabetes can also affect hematological parameters.

The results obtained in this study, however, contradict studies conducted in the rural CAR Dzanga-Sangha region in 2020, which showed asymptomatic malaria infections in children with no statistically significant correlation between hemoglobin levels and malarial infection [28]. Our study confirms that malaria-associated anemia contributes to the symptomatic form of malaria compared with this previous study. Anemia is the first symptom noted in severe malaria (about 60%) in pediatric wards in Bangui, before convulsion [11].

Our study showed an increase in the leukocyte count, including neutrophils, in contrast to a study in Morocco where the leukocyte and neutrophil counts were normal in all patients [29]. However, this difference can be attributed to the high level of transmission in endemic areas compared with non-endemic areas, which affects the dynamics of the host response. Neutrophils, the most numerous types of leukocytes, contribute to immunity against malaria through the elimination of parasites by phagocytosis, producing reactive oxygen species and releasing neutrophilic extracellular traps (NETs) [30]. However, neutrophils may also be involved in the development of complications of malaria via the release of toxic granules and NETs, hence the functional duality of neutrophils. Finally, the results obtained in this study also contrast with studies conducted in Ghana and Ethiopia, which both reported thrombocytopenia as a major hematological change in malaria cases [20] [31].

There was no significant difference in parasite density according to hematological parameters and age group, the results obtained contrast with study conducted in Ethiopia, which both reported parasitemia there were statistically significant differences to hematological parameters [32]. A study conducted in Nigeria has shown that, malaria parasitemia has been shown to have effects on some haematological parameters from this study while some haematological parameters are more predictive of malaria infection than others. Lymphopenia is however more indicative of hyperparasitemia, neutrophil, monocyte and basophil counts do not have any significant relation with malaria parasitemia [33]. Linear regression also

showed a linear relationship between parasite density and the various haematological parameters [34].

Our study confirms previous studies that showed hematological changes associated with malarial infection can vary depending on the following factors: the level of malaria endemicity, background hemoglobinopathy, demographic factors and malaria immunity. One study in India revealed that hemoglobin concentrations are significantly lower in low- than in high malaria-endemic districts [34]. The hemoglobin concentration, in terms of anemia, remains the most common factor associated with malaria. This study contributes to the body of literature covering the effects of malaria infection on the hematological profile of infected persons.

Several limitations of this study should be acknowledged. First, the study site does not adequately reflect the general population of Bangui, given that it is primarily utilized by individuals of medium to high socioeconomic status. The causes of nutritional deficiency and individuals infected with other conditions were not ruled out in the present study. The lack of hematological reference ranges for children and adults from the CAR represents one weakness of this study. The retrospective nature of the analysis, which relies on existing historical data, leads to selection bias. Finally, our study would have benefitted from being carried out in a health center or hospital setting to assess the clinical aspects of the disease and particularly to identify the form of the malaria infection (asymptomatic, uncomplicated or severe). Future multicenter studies involving larger and more diverse sites, with the anemia thresholds and hematology reference intervals by age and sex and using both cross-sectional and prospective designs, are needed to further validate these findings and enhance their generalizability.

5. Conclusion

This study revealed several aspects and facets of malaria in the CAR, including a shift of the at-risk age group from the under 5 years to the 5 - 14 years age group. Study participants infected with malaria demonstrated vital changes in most hematological parameters, showing anemia and leukocytosis, including neutrophilic leukocytosis. Hematological abnormalities are common features of *P. falciparum* malaria, but the different parameters can vary between populations and areas. Malaria patients should be assessed for the presence of these hematological abnormalities and need to be managed in a timely manner to improve their prognosis. Parasite density is an indicator of infectious burden; however, its clinical significance depends largely on the individual's immune status, which is influenced by the intensity of local malaria transmission. Finally, future studies should adopt a cross-sectional study approach and strict inclusion criteria with larger sample sizes to investigate and refine these different observations.

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Ethics Statement

The study was conducted in accordance with the guidelines and requirements of the Ministry of Health and Population, including the current standard protocol for the management and routine monitoring of severe malaria, without any additional specific interventions. Consequently, an exemption from ethical approval was granted by the General Directorate of Scientific Research and Technological Innovation of the Ministry of Higher Education, Scientific Research, and Technological Innovation (Research Authorization No. 026/MESRSIT/DGRSIT/25). Nevertheless, all participants were assured of the confidentiality and anonymity of their data, which were securely stored and used exclusively for research purposes.

Data Availability

The raw data supporting this study are available from the authors upon request.

Conflicts of Interest

The authors declare that they have no conflict interest.

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List of Abbreviations

CAR: Central African Republic;
RBC: Red blood cell;
RDT: Rapid diagnosis test;
WHO: World Health Organization;
MCH: Mean corpuscular hemoglobin;
MCV: Mean corpuscular volume;
NETs: Releasing neutrophilic extracellular traps.