

## Influence of ABO Blood Groups on *Plasmodium falciparum* Parasitaemia and Malaria Clinical Types in Outpatients in a Government Hospital of Douala, Cameroon

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### Abstract

*Falciparum* malaria is a major cause of morbidity and mortality worldwide. Plasmodium infected patients carry a wide range of parasitic loads and exhibit asymptomatic, mild or severe malaria. Among host intrinsic factors which likely influence development of malaria type, controversies remain on the relationship between malaria infection and ABO blood groups types. This cross-sectional study was designed to investigate any relationship between ABO blood types, Plasmodium loads and clinical type of malaria among outpatients received in Bonassama hospital.

Each outpatient who volunteered for the study was examined, tested for ABO blood types and malaria parasites carriage. Data were statistically analyzed for any association.

Of 375 *Plasmodium falciparum* infected patients included, ABO blood group frequency was 45.3% (O), 25.3% (A), 21.6% (B), 7.8% (AB). All ABO blood groups harboured predominantly light intensities of *falciparum* infections; however high intensities of infections were significantly frequent in blood group A. Gender did not significantly influence *P. falciparum* infection prevalence. Infection prevalence was significantly high in less than 15 years blood group A patients. *P. falciparum* infection prevalence was not significantly different among blood group patients ( $p > 0.05$ ). Although all ABO blood group patients exhibit different clinical malaria types, severe-like malaria symptoms were exhibited more frequently among blood group A and group B patients. Blood group O patients exhibited predominantly asymptomatic and uncomplicated malaria.

No significant association was found between Plasmodium loads, gender and age groups. Under five year blood groups A and B patients were likely more affected by severe malaria whereas blood group O patients suffered predominantly from mild and symptomless malaria.

Findings from this study demonstrated that in Douala, young blood group A and B patients were more predisposed to high intensities of *P. falciparum* infections and severe malaria whereas blood group O exhibited mostly light infections associated to mild and asymptomatic malaria.

**Keywords:** *Falciparum* malaria; ABO blood groups; Relationship; Parasitaemia; Clinical stages

### Introduction

Malaria is a highly life-threatening parasitic disease caused by inhabiting red blood cells parasites named *Plasmodium* sp. where they multiply often lead to the burst of the host cell. An estimated 2 billion persons were at risk of infection to malaria worldwide up to 2014 of whom 84% live in sub-Saharan Africa [1-3]. This parasitic disease is a major cause of morbidity and mortality worldwide, and the infective parasite is particularly virulent among young African children, pregnant women and all age groups travellers originating from non-endemic countries [1,4]. Of the five species which infect man, *Plasmodium falciparum* is the most deadly specie. The virulence of *P. falciparum* has been associated with the capacity of the infected RBCs to surround themselves with uninfected red blood cells leading to rosetting of cells and development of severe-like malaria [5-8]. Infected subjects can develop a wide range one or more symptoms which determine one of the above clinical stages of malaria: asymptomatic malaria, mild malaria or severe malaria. The occurrence of each other clinical form of malaria has been usually associated to some risk factors either independent or related to human. Ethnicity, parasitaemia and a history of previous clinical malaria has been demonstrated to significantly influence the outcome of WHO-defined severe *falciparum*

malaria [4]. History of previous malaria infection is thought to enhance a partial immunity which likely downregulates the outcome of clinical malaria. Such regulation by the immune system occurs much rapidly as the infections are frequent like in stable malaria transmission, and later in teenagers in seasonal malaria transmission areas. Human intrinsic risks factors have been also pointed to influence the susceptibility to malaria and development of either malaria stage. These intrinsic factors include sickle cell trait (HbAS),  $\alpha$ -thalassaemia, Glucose-6-phosphate dehydrogenase (G6PD) deficiency, erythrocyte variants as well as ABO blood group types. These factors act either by regulating parasites loads or occurrence of clinical forms of malaria. Age and acquired immune responses have long been the most frequent human-related determinants of the host susceptibility to clinical malaria and infection. Sickle cell trait (HbAS) and  $\alpha$ -thalassaemia are proved to protect

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against severe and fatal malaria but none has effect on asymptomatic parasitaemia [9]. In Mali, Fulani group with rare less 202A mutation on G6PD were shown less susceptible to malaria than the Dogon group who have a high mutation of the 202A haplotype [10].

Concerning the ABO blood group types, there is a controversy among studies from different countries upon association between blood group and malaria. Blood group O individuals were relatively protected from severe malaria than those of other blood groups [11-13]. Blood group O patients from Ethiopia were demonstrated less prone to severe malaria as compared to patients with other blood groups [14]. Significant associations between blood group and *P. falciparum* malaria have been reported from cross-sectional and case control studies in Brazil [15], Gabon [16], India [17] whereas studies in Colombia, India, Sudan and Nigeria did not find any association between malaria and ABO blood group [18-22]. Despite these controversies, non-O blood groups emerged in most of the studies as significant risk factors for life-threatening malaria through the mechanism of enhanced rosette formation [12] and then blood group A and blood group B have been demonstrated as *Plasmodium* co-receptor in the rosetting-forming process thus enhancing occurrence of severe malaria [8].

Due to such controversies on the relationship between malaria infections outcomes and blood groups, and also due to the fact that such data are scarce in Cameroon, this study has been undertaken with the aim to determine the relationship between ABO blood group, malaria parasitaemia and clinical form of malaria among different age group patients who attended an hospital in Douala city of Cameroon.

## Patients and Methods

### Study type and place

This was a cross-sectional study carried out from November 2013 to May 2014 at the Bonassama district hospital for recruitment of patients and the Laboratory of the Faculty of Medicine and Pharmaceutical Sciences for laboratory analysis.

### Ethics

This research study was carried out in compliance with the Helsinki Declaration. The protocol of the study was approved by the Littoral Regional Public Health Delegation in Cameroon and the Ethic Committee of the University of Douala. Patient recruitment started after we obtained a written authorization letter from the director of the Bonassama district hospital. Douala town is located in a stable malaria transmission area.

### Data collection

During the study period, any outpatient who attended the Bonassama District Hospital was asked to volunteer for the study. For all patients who accepted to participate, the study protocol was read to the patient then each patient who met the study criteria was included in the study. The study inclusion criteria were as follow: i) be a Cameroonian irrespective to gender; ii) age at least one year; iii) have reside continuously in the Douala town or outskirts for at least one month; iv) not have taken any antimalarial medication within the four weeks which preceded the inclusion visit; v) not have any chronic or immunodeficient known disease; vi) sign the study informed consent. Individual informed consent was obtained from adults patients but for patients aged less than 15 years and adult patients who were unable to answer to the study questionnaire, the study protocol was read to his legal parent or guardian, then the study informed consent was obtained

from the later. Each eligible patient underwent a questionnaire, a clinical examination and blood tests. Questionnaire sought to collect data regarding age, sex, residence duration in the area, and complains as fever, headache, joints pains. Blood laboratory analysis was performed for ABO blood group testing together malaria parasites detection.

**Clinical examination:** The clinical examination aimed to search for symptoms related to mild as well as severe malaria as defined by WHO guidelines [23,24]. The main mild malaria sign investigated was hyperthermia at the inclusion visit recorded through body temperature measurement. All malaria parasites carrying patients confirmed by laboratory analysis were considered as malaria cases and classified as “severe malaria patients” if they were positive for at least one of the severe malaria criteria as outlined by the World Health Organization [23,24], “mild malaria patients” if though having clinical sign of malaria they were severe-like malaria symptom-free, or “asymptomatic” if they were malaria-like symptom-free.

**Malaria parasites diagnosis:** Blood *Plasmodium falciparum* asexual stages were detected and counted using stained thick and thin blood smears performed from patient freshly collected whole blood. Blood smears were air dried and stained with 10% Giemsa solution according to routine standards [25,26]. The stained smears were examined under a light microscope using 100 × oil immersions by an experienced laboratory technician. Slides were cross-checked by the study investigators. Parasitaemia was calculated per 500 white blood cells (WBCs) assuming that each outpatient had a mean 7500 WBCs/ $\mu$ l of blood [25,26]. Parasitic loads were classified according to asexual stages load as follow: 1) light for parasitic load less than 2000 trophozoites/ $\mu$ l of blood; 2) moderate if the parasitic load ranged between 2000 and 5000 asexual stages / $\mu$ l of blood; 3) high for parasitic load over 5000 asexual stages/ $\mu$ l of blood.

**Blood groups testing:** ABO blood groups were typed by agglutination using commercial antisera as previously described [27,28]. Two drops of whole blood were placed in two different places of a grease-free clean glass slide. A drop of antiserum for blood group A was applied to one of the blood spot and a drop of blood group B antiserum was added to the second blood spot. Each blood spot and the antiserum were mixed with a sterile discardable applicator stick. The slide was then tilted to detect for agglutination and the result recorded accordingly [27,28].

Data obtained were analyzed for any association between *P. falciparum* intensity of infection, clinical stage and blood group by chi-square ( $\chi^2$ ) statistical method. Statistically significance was considered at 95% level of confidence and P value less than 0.05.

## Results

A total of 375 *Plasmodium falciparum* infected patients were included in the study. Female patients represented 58.67% of the sample. Mean age of the patients was 25 years ( $\pm$  19.2 years) range (one month to 84 years). ABO blood groups frequency occurrence was 45.3%, 25.3%, 21.6% and 7.8% for O, A, B and AB respectively.

The overall *Plasmodium* infection prevalence was 37.1% as detected by microscopy examination. Mixed infection with other *Plasmodium* specie was not included in this study.

### *Plasmodium* infection prevalence according to ABO blood groups

As indicated in Table 1, *Plasmodium* infections occurred most

frequently in blood group O patients whereas blood group AB infected patients were less frequent. However, *Plasmodium* infection prevalence was not significantly different among blood group patients ( $\chi^2=2.38$ ; df3;  $p>0.05$ ). *Plasmodium* infection was diagnosed in 34.1%, 44.2%, 37%, and 31% of blood group O, A, B and AB patients respectively. Specific infection by *P. falciparum* occurred in 33.5%, 43.1%, 37% and 31% of blood groups O, A, B and AB patients respectively. The *P. malariae* infections cases occurred only in blood groups O and A patients.

Among infected subjects, over 15 years old patients were more representative than younger one (66.9% vs 33.1%). Under 5 years children represented 23% of infected patients.

Within age groups, less than 15 years blood group A patients had highest infection frequency than those over 15 years old. There was no significant difference between less than 15 years infected subjects compared to those aged over 15 years among blood group O patients. However, in blood groups A, B and AB subjects, there appeared a significant difference in *Plasmodium* infection between patients over 15 years old and younger ones. Sex did not significantly influence infection by *Plasmodium falciparum* ( $p>0.05$ ) (Table 2).

### Plasmodium falciparum asexual stage intensities of infection

Parasitic loads recorded ranged between 375 trophozoites/ $\mu$ l of blood and 315 000 trophozoites/ $\mu$ l of blood. As shown in Table 3 below, all blood groups patients carried predominantly light intensities of infection. However, blood group A patients had the highest frequency of high asexual stages loads (44.4%). There was no significant difference between ABO blood groups types and heavy parasitic loads carriage ( $p>0.05$ ). However, blood group A and blood group AB patients were likely to harbour high *Plasmodium* asexual stage loads than the other blood group patients.

Light and heavy infections represented 83.4% and 6.5% of *Plasmodium* infected subjects respectively. Moderate infection accounted for 10.1% of *Plasmodium* carriers. One 7 years old blood group AB female patient harboured more than 50000 asexual stages/ $\mu$ l of blood.

Light *Plasmodium falciparum* asexual stage load (less than 2000 trophozoites/ $\mu$ l of blood) was predominant (76.3%) among included subjects. 26.4% of these light parasitic loads carrying patients were under 15 years old, and 73.6% of them were over 15 years old. One 7 years

old patient had a parasitic load over 50 000 trophozoites/ $\mu$ l of blood.

### Clinical trends of Plasmodium falciparum carrying patients

At inclusion, 48% of *Plasmodium* infected had at least a malaria-like symptom and the remaining patients were symptom-free. Among those with a malaria-like symptom, 86.7% were feverish.

Among *P. falciparum* infected subjects 40.3% were asymptomatic, 48.9% had uncomplicated malaria and 10.8% showed at least severe-like malaria symptom. As shown in Table 3, symptom-free and mild malaria patients were most common groups in blood group O, B and AB patients. Severe malaria was more frequent among blood group A patients and blood group AB patients than mild and symptomless malaria. Blood group A patients were likely to develop more severe malaria symptom than the other blood groups. There was however no significant difference between blood group types and the clinical type of malaria developed by the patients ( $p>0.05$ ). Analysis of prevalence of clinical malaria types with respect to age groups and blood group indicated that severe malaria occurred mainly in less than 15 years infected subjects. Within blood group A and blood group B infected subjects, severe malaria occurred mainly in under 15 years subjects, whereas only under 5 years blood group AB suffered from complicated malaria. Both younger and older blood group O patients suffered from severe malaria.

Asymptomatic *P. falciparum* infections and uncomplicated malaria occurred at all age in blood groups O, blood group A and blood group B patients. However, prevalence of asymptomatic and uncomplicated malaria was significantly higher in less than 15 years blood group A and blood group B *Plasmodium* infected patients than in older subjects ( $p<0.05$ ).

### Discussion

This study aimed to find any association between intensities of asexual stages *Plasmodium falciparum* infection, ABO blood group types and clinical features of malaria among outpatients and inpatients received at Bonassama district hospital in Cameroon. This hospital is located in Douala town which is known as area of stable malaria [29]. The recruitment period extended from March to May which corresponds to the rainy season and therefore favourable for malaria cases. Diagnostic techniques used namely thick plus thin blood smears

Blood group	Specific infections				Gender				Age (years)					
					Male		Female		0-4		5-14		≥ 15	
	N	Global	<i>P. falciparum</i>	<i>P. malariae</i>	N	%	N	%	N	%	N	%	N	%
O	170	34.1	33.5	0.6	71	30.1	99	36.4	36	30.5	12	33.3	122	35.2
A	95	44.2	43.1	1.0	41	46.3	54	42.6	24	50.0	12	75.0	59	35.6
B	81	37.0	37.0	0.0	32	31.2	49	40.8	26	34.6	5	0.0	50	42.0
AB	29	31.0	31.0	0.0	11	27.3	18	33.3	4	0.0	2	50.0	23	34.8
	375	37.1	36.5	0.53	155	34.8	220	38.6	90	35.5	31	45.2	254	36.6

Table 1: Plasmodium infection prevalence in blood group participants according to Plasmodium species, gender and age. N = sample size examined.

Asexual stages / $\mu$ l of blood	ABO blood groups				Age groups (years)		
	A	B	AB	O	< 5	5-14	≥ 15
1 – 2000	35.8	33.3	27.6	27.6	31.1	32.2	30.7
2001 – 5000	4.2	1.2	0.0	5.3	2.2	6.4	3.9
> 5000	4.2	2.4	3.4	1.2	2.2	6.4	1.9
TOTAL	44.2	37.0	31.0	34.1	35.5	45.2	36.6

Table 2: Frequency of P. falciparum asexual stage loads according to ABO blood groups and age.

Blood group	Prevalence of clinical malaria type												Frequency of clinical malaria type									Total
	Asymptomatic				Uncomplicated				Severe				Asymptomatic			Uncomplicated			Severe			
	N	0-4	5-14	≥15	N	0-4	5-14	≥15	N	0-4	5-14	≥15	0-4	5-14	≥15	0-4	5-14	≥15	0-4	5-14	≥15	
O	23	16.7	33.3	10.6	31	30.5	50.0	11.5	3	5.5	0.0	0.8	4.3	2.9	9.3	7.9	4.3	10.1	1.4	0.0	0.7	41.0
A	14	29.2	16.7	8.5	21	37.5	41.7	11.9	6	16.7	16.7	0.0	5.0	1.4	3.6	6.5	3.6	5.0	2.9	1.4	0.0	29.5
B	18	11.5	60.0	24.0	12	7.7	0.0	20.0	4	11.5	20.0	0.0	2.1	2.1	8.6	1.4	0.0	7.2	2.1	0.7	0.0	24.5
AB	1	0.0	0.0	4.3	4	0.0	0.0	17.4	2	50.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	2.9	1.4	0.0	0.0	5.0
Total	56	17.7	29.0	12.2	68	24.4	35.5	13.8	15	12.2	9.7	0.4	11.5	6.5	22.3	15.8	7.9	25.2	7.9	2.1	0.7	100
	$\chi^2=5.45$ ; df6				$\chi^2=11.9$ ; df6				$\chi^2=5.45$ ; df6				$\chi^2=15.01$ ; df6			$\chi^2=11.7$ ; df6			$\chi^2=2.08$ ; df6			

Table 3: Prevalence and frequency of clinical malaria form according to blood group and age. N = Plasmodium falciparum infected cases recorded.

and Rapid Diagnostic tests were the most recommended for malaria diagnosis and better management of malaria cases [1,25,24,30]. ABO blood group types were determined as indicated by the manufacturer leaflet using the Beth-Vincent method which the most common approach used in health facilities in Cameroon.

The distribution of patients according to ABO blood group types was almost similar to the overall known distribution in the African population and elsewhere with a predominance of blood group O subjects [31,32].

Plasmodium falciparum infections occurred in all blood groups patients. Since there appeared no significant difference in infection prevalence, the blood groups seemed not to influence infection by malaria parasites. As usually described in most epidemiological data in Cameroon and most malaria endemic areas, P. falciparum was the predominant specie occurring in 98.6% infected patients. P. falciparum infections occurred in all blood group patients whereas P. malariae were recorded only in blood groups O and A patients. Predominance of P. falciparum in this study corroborated with previous reports in Cameroon which pointed the latest specie to represent over 90% of the overall Plasmodium infection in this country alongside P. malariae and P. ovale [29].

Blood group O patients had the highest infection prevalence compared to other blood group patients. This result may be due to highest sample size of blood group O in the study. Blood group O subjects therefore were likely to be encountered by female Anopheles sp flies than other blood group subjects.

Concerning P. falciparum asexual stage loads, it was predominantly parasites of light intensity in all blood groups. However, frequency of light intensity of infection was higher in blood group O and decreased as intensity of infection became heavy. We didn't find any previous data concerning ABO blood group types and P. falciparum asexual stage loads. Heavy intensities of infection were most common among infected blood group A patients.

Concerning clinical malaria type, all blood group patients presented predominantly mild and asymptomatic malaria. However, since blood group A and blood group B infected patients presented the greater number of severe-like malaria, these blood groups likely predispose to development of severe symptom of malaria. This observation corroborates previous data from Ethiopia which reported a predominance of severe malaria among blood group A P. falciparum infected patients than other blood groups [14]. Previous studies have already pointed the ABO blood group type in rosetting process which is the dominant process of the pathogenesis leading to severe malaria [21]. Recent studies among in Malian children demonstrated rosetting (the well-known Plasmodium falciparum parasite virulence factor) to occur more frequently in Plasmodium falciparum parasites isolates from

severe malaria patients compared with non-severe hyperparasitemia and uncomplicated malaria controls [33]. In order to identify the receptors and some mechanisms which govern the interplay between malaria parasites and blood group antigens, biochemical investigations pointed blood group A and blood group B antigens as coreceptors of Plasmodium parasites in the development of severe malaria by enhancing rosetting formation in P. falciparum infected red blood cells, a process which did not occur frequently with blood group O antigens [8]. Also, concerning the contribution of ABO blood group types in the development of severe malaria, rosette-forming adhesion molecules rosetting have been identified in all ABO blood group red blood cells with a marked preference for group A over group B, which in turn is preferred to group O red blood cells [34,35]. Observations in this study showing blood group A and blood group B P. falciparum infected to develop more frequently severe malaria than blood group O patients were therefore in accordance previous observations. However, since within P. falciparum infected blood group A and blood group B severe malaria symptoms occurred more frequently in younger subjects, this suggested that absence of immunity may still be a major factor which modulates development of clinical malaria in endemic areas. This high susceptibility of young patients to severe malaria confirmed their high vulnerability to malaria and call for more consideration of this age group in the malaria control strategy by the World Health Organization and malaria endemic countries health ministries in Africa [1-3,30].

Results from this study also indicated that P. falciparum infected blood group O patients developed predominantly asymptomatic and uncomplicated malaria, and less frequently severe malaria symptoms. Such data agree with previous reports which pointed blood group O Malian children to be less prone to severe malaria than other blood groups subjects [11]. This less susceptibility of blood group O to severe malaria may be explained with reports from studies from studies in pregnant women which showed significant association between blood group O and increased placental malaria infection in primiparae and multiparae compared to other ABO phenotypes, a process which may modulate the pathogenesis of malaria in offspring [35]. A recent study in primiparae living in an area of high malaria endemicity of Ghana also suggested the protective influence of blood group O to severe malaria against P. falciparum infection [13].

## Conclusion

Data from this study indicated that all ABO blood groups were likely to be infected by Plasmodium and developed asymptomatic, uncomplicated as well as severe malaria. However, blood group O infected subjects carried mostly light infections intensities and developed more frequently asymptomatic and mild malaria whereas severe malaria was exhibited more frequently by young blood group A and blood group B infected patients. The study emphasized that occurrence of heavy intensities of P. falciparum infections and high

frequency of severe malaria recorded in blood group A and blood group B patients were more related to young age of patients.

#### Conflict of Interests

The authors of this manuscript declare that they have no conflict of interest concerning this study.

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#### Contribution of Authors

ASE and TK designed the study and were involved in all aspects of the study, data collection, analysis, interpretation and in writing of the manuscript. EKK, GPN, EHMM participated in data collection. ASE supervised the study. All authors read and approved the manuscript.

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