

Original Research Article

Strong linear correlation between parasite density and ABO-blood group among patients with malaria parasite infection

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ABSTRACT

Background: Analysis of the dynamics of *Plasmodium falciparum* infection as it relates to the ABO blood group system could expand our understanding of malaria pathology and further global efforts in addressing the scourge of malaria disease. This study seeks to examine the association between malaria infection and parasite density in relation to the ABO blood group system.

Methods: 298 patients (Age Mean±SD = 28.8±9.16) were selected at random and screened for malaria parasite infection and parasite density quantification using the thick blood film method. Pearson correlation analysis was used to determine relationship between parasite density and blood group.

Results: 88 (29.5%) patients tested positive to malaria parasite infection. Patients with blood group O recorded the highest number of positive cases (56.81%) and the least was found among those with blood group AB (3.42%). 93.18% of patients that tested positive were rhesus positive while 6.82% were rhesus negative. 40.90% of patients with mild parasitaemia and 10.23% of patients with moderate parasitaemia were of blood group O+. Pearson correlation coefficient was strong, direct and linear ($r = 0.9184$; $r^2 = 0.8434$).

Conclusions: This study has shown that there is a strong correlation between parasite density per microliter of blood in relation to the ABO-blood group type of individuals diagnosed with malaria parasite infection.

Keywords: Blood group, Correlation, Malaria, Parasite density, *Plasmodium falciparum*

INTRODUCTION

Malaria is caused by *Plasmodium spp.* transmitted, in most cases, by female Anopheles mosquitoes (malaria vectors). Five (5) Plasmodium species are recognized to cause malaria in humans out of which two (2); *P. falciparum* and *P. vivax*, pose the greatest threat. *P. falciparum* is the most prevalent malaria parasite on the African continent. It is responsible for most malaria-related deaths globally. *P. vivax* is the dominant malaria parasite in most countries outside of sub-Saharan Africa. The intensity of transmission depends on factors related

to the parasite, the vector, the human host, and the environment.¹

Most malaria cases and deaths occur in sub-Saharan Africa. However, South-East Asia, Latin America and the Middle East are also at risk. According to the latest WHO estimates, there were 212 million cases of malaria in the year 2015 and 429 000 deaths.¹ 97% of the estimated 160 million population of Nigeria are at risk of *Plasmodium falciparum* infection.² Nigeria has the highest number of malaria cases and deaths (estimated at 51 million cases and 207,000 deaths) annually, more than any other

country in the world and approximately 25% of the total malaria burden within Africa.¹

Plasmodium falciparum infects erythrocytes of any age with the potential of development of high-grade parasitaemia.³ Due to the rapid multiplication of this parasite, the parasite count can increase up to 20-fold over a period of 48 hours without treatment.⁴ In the clinical setting, the level of parasitaemia is useful as one of the criteria in defining “severe *P. falciparum* malaria” and to monitor the effect of anti-malarial therapy.⁵ Peripheral blood parasite density can be used to calculate malaria-attributable disease in slide-positive severe malaria in African children.¹ It has been suggested that the case definition for severe malaria is improved by applying a parasite density threshold (2500/μl in the Kenyan setting).⁶

Numerous studies have been conducted to ascertain the association between genetic markers and malaria, since the discovery that sickle-cell hemoglobin offers protection against infection by *P. falciparum*. A broad range of available literature suggests that the origin, distribution and relative proportion of ABO blood groups in humans may have been directly influenced by selective genetic pressure from *Plasmodium falciparum* infection.⁷

The correlation between severity of malaria infection to the patient’s blood group has been of recent interest in the quest for answers to the factors influencing the clinical course of the disease. The observation that human erythrocytes lacking the Duffy blood group antigens are refractory to invasion by *P. vivax* parasites indicate the usefulness of studying the association of blood group with malaria.⁸ This study seeks to examine whether or not, there exist a correlation between parasite density and the blood group type of patients diagnosed with malaria parasite infection.

METHODS

Study area

The study was conducted in Maiduguri, the capital of Borno State. The city is located in the North-Eastern part of Nigeria which lies within latitude 11.15°N and longitude 30.05°E in the sudano-sahelian savannah zone with a dense population that are mostly crop farmers, fishermen, herdsman and traders.⁹ Based on the national census conducted in 2006, Borno State has a population of 4 151 193.¹⁰

Study population

The target population for the study include in-patients and out-patients attending the University of Maiduguri Teaching Hospital. The hospital is a tertiary care, 530 bed facility serving a population of over 20 million in the North-Eastern sub-region of Nigeria, comprising six States (Borno, Bauchi, Yobe, Adamawa, Taraba and

Gombe) as well as a sizeable number across the borders of Cameroon, Chad and Niger Republic.¹¹ Ethical clearance was obtained from the management of the University of Maiduguri Teaching Hospital and the consent of patients was obtained before sample and data collection

Sample collection and processing

Using sterile syringe and needle, two milliliters (2ml) of venous blood was collected from 298 patients (Age Mean±SD = 28.8±9.16; Male:Female =9.6:1) at random. Blood samples collected were dispensed into Ethylene-Diamine-Tetra-acetic Acid (EDTA) containers, properly labeled and transferred to the laboratory for further analysis.¹²

Blood group determination

The ABO blood group of each patient was determined using cell grouping Antisera.¹² Full time is allowed for the detection of weak reactions and results were interpreted accordingly.

Test for malaria parasite infection

Thick blood film method was used in the analysis of blood samples for malaria parasite and parasite quantification as well. A drop of blood (about 15 mm) was placed on a clean grease-free microscope slide and spread to make a thick smear. The slide was labeled, and the thick blood smear was allowed to air-dry with the slide placed in a horizontal position.

Holding the slide with the dried thick film facing downwards, the slide was dipped into Field’s stain A for 5 seconds. Excess stain is then drained off and washed gently for about 5 seconds in clean water. The excess water is drained off and the slide is dipped into Field’s stain B for 3 seconds. Excess stain is drained off and then washed gently in clean water. The back of the slide is then wiped clean and placed upright in a draining rack for the film to air-dry. When the thick film is completely dry, a drop of immersion oil is applied to an area of the film which appears mauve coloured (usually around the edges) and examined using x10 objective lens and x100 objective for higher magnification.¹²

Parasite density determination

Parasite determination was done by counting the number of asexual forms of *Plasmodium falciparum* parasites against at least 100 leucocytes and 200 leucocytes for definitive count. The number of asexual parasites was calculated using this formula:

$$\text{Parasites}/\mu\text{L} = \frac{\text{No of asexual parasites} \times 8000}{\text{leucocytes}/200 \text{ leucocytes}}$$

The degree of parasitaemia was graded thus:

- 1-999/ μ L as mild or +,
- 1000-9999/ μ L as moderate or ++
- >10000/ μ L as severe or +++¹³

Statistical analysis

Data collected were analyzed using IBM SPSS Statistics 2.0 software.¹⁴ Data were presented as frequencies and percentages in tables and figures. Pearson correlation coefficient (r) and Coefficient of determination (r²) were used to determine the relationship between variables

RESULTS

Relationship between patient demographics and malaria parasitaemia

A total of 298 samples were processed out of which 88 tested positive to malaria parasite infection. This gives an infection rate of 29.5%. Patients in the age category of

20-40years were the most infected (70.44%) and the least were those within the age category of >40years old (13.64%). The rate of infection is higher among males (93.16%) than females (6.84%) (Table 1).

Relationship between blood group, Rhesus factor of patients and malaria parasitaemia

Rate of infection in relation to blood group of patients has shown that patients of blood group O had the highest (56.81%) while the least was found among patients of blood group AB (3.41%). A significant rate of infection was observed among patients of blood group O and within the age category of 20-40years (40.91%) but a very low rate was observed among patients of blood group AB and within the age category of <20years and >40years (0.00% respectively). On sex versus blood group basis, male patients of blood group O recorded the highest infection rate of 53.40% and the least was found among female patients of blood group AB (0.00%).

Table 1: Percentage Occurrence of malaria parasitaemia in relation to blood group and demographic characteristics of patients examined.

Demographics	Blood groups (n=88)				Total (%)
	A (%)	B (%)	AB (%)	O (%)	
Age (yrs)					
<20	4 (4.55)	3 (3.41)	0 (0.00)	7 (7.95)	14 (15.91)
20-40	12 (13.63)	10 (11.36)	3 (3.41)	36 (40.91)	61 (69.32)
>40	4 (4.55)	2 (2.27)	0 (0.00)	7 (7.95)	13 (14.77)
Total (%)	20 (22.73)	15 (17.04)	3 (3.41)	50 (56.81)	88 (100)
Sex					
Male	18 (20.45)	14 (15.90)	3 (3.41)	47 (53.40)	82 (93.18)
Female	2 (2.27)	1 (1.14)	0 (0.00)	3 (3.41)	6 (6.82)
Rhesus Factor					
Positive	19 (21.59)	15 (17.04)	3 (3.41)	45 (51.14)	82 (93.18)
Negative	1 (1.14)	0 (0.00)	0 (0.00)	5 (5.68)	6 (6.82)

Relationship between patient’s blood group/rhesus factor and malaria parasitaemia indicate that patients of O+ recorded the highest (51.13%) followed by A+ (21.59%), B+ (17.04%), O- (5.68%), AB- (3.41%), A- (1.14%) and the least was found among B- and AB- (0.00% respectively). 93.18% of the 88 malaria positive patients in this study were rhesus positive while 6.82% were rhesus negative (Table 1).

Parasite density in relation to patients’ blood group and Rhesus factor

Comparative analysis between parasite density and blood group/rhesus factor of patients revealed that out of the 71 patients with mild parasitaemia, 40.90% were from blood group O+, which was the highest and least was found among group B- and AB- (0.00% respectively). 73.86% were rhesus positive while 6.82% were rhesus negative.

Table 2: Parasite density in relation to blood group/Rhesus factor of patients examined.

Blood group	Parasite Density (%) (n=88)		
	1-999/ μ L	1000-9999/ μ L	>10 000/ μ L
A+	17 (19.32)	2 (2.27)	0 (0.00)
A-	1 (1.14)	0 (0.00)	0 (0.00)
B+	11 (12.50)	4 (4.55)	0 (0.00)
B-	0 (0.00)	0 (0.00)	0 (0.00)
AB+	1 (1.14)	2 (2.27)	0 (0.00)
AB-	0 (0.00)	0 (0.00)	0 (0.00)
O+	36 (40.90)	9 (10.23)	0 (0.00)
O-	5 (5.68)	0 (0.00)	0 (0.00)
Total (%)	71 (80.68)	17 (19.32)	0 (0.00)
Rhesus factor			
Positive	65 (73.86)	17 (19.32)	0 (0.00)
Negative	6 (6.82)	0 (0.00)	0 (0.00)

A total of 17 (19.32%) of patients had moderate parasitaemia and patients with blood group O+ recorded the highest (10.23%) and the least was found among group A-, B-, AB- and O- (0.00% respectively). All patients with moderate parasitaemia were rhesus positive. There was no case of severe parasitaemia recorded in this study (Table 2).

Correlation analysis between parasite density and blood group of patients studied

The Pearson correlation coefficient (r) was calculated and from Table 3:

$$r = 0.9184.$$

This indicates a very strong linear correlation between parasite density and the blood group of patients. And the coefficient of determination (r^2) is calculated:

$$r^2 = (0.9184)^2 = 0.8435$$

Thus, it can be deduced that the variability in blood group of patients accounted for 84% of the variability observed in parasite density among patients examined.

Table 3: Correlation analysis of parasite density with respect to the blood group of patients examined.

Correlation analysis	Parasite density versus Blood groups
Pearson Correlation Coefficient (r)	0.9184
Coefficient of determination (r^2)	0.8435
N	8

DISCUSSION

The persistence of malaria infection is a serious public health problem in many parts of the world, particularly countries in sub-Saharan Africa. It is therefore important to identify those factors that contribute to host susceptibility to infection with a view to develop the capacity to address the burden of the disease. The low parasite prevalence (29.4%) recorded in this study may not be unconnected with the fact that the study was conducted during the dry season when malaria infection is generally low compared to the end of rainy season, when the infection rate is quite high.¹⁵

Studies indicating very low prevalence rates were reported in Ibadan and Maiduguri with 10.0% and 6.0% respectively.^{16,17} Contrary findings were reported in Warri and Owerri, which revealed a prevalence rate of 79.3% and 77.4% respectively.^{18,19} Such differences can be attributable to climatic and regional differences in the distribution of malaria parasite infection.

The rate of infection in this study, was highest among males (93.16%) compared to females (6.84%). Adults

between the ages of 20-40years recorded the highest rate of parasitaemia (70.44%) and older patients above 40 years of age recorded the least (13.6%).

The high level of parasitaemia observed among patients of blood group O (56.81%) in this study is in agreement with other studies conducted in Nigeria where a prevalence rate of 63.83% and 74.5% were reported.^{20,21} This could be explained partly by the fact that majority of the population from which the sample was drawn were of blood group O.²²

The variation may also be ascribed to the feeding habits of the vector species. It has been reported that some people are more prone to mosquito bites than others and under laboratory conditions, *Anopheles gambiae* seems to recognize blood groups and to feed preferentially on those with blood group O.^{23,24} Some researchers have attributed an intrinsically low infection rate among individuals with blood group O. This is not always the case, as results of this study and similar ones have shown. It can be said that malaria morbidity reflects differences in the *Plasmodium* specie involved, host immune status, climatic variation, hemoglobin and other factors.

Relationship between parasitaemia and Rhesus factor indicates that 93.18% of malaria positive patients were rhesus positive while 6.82% were negative. Similar findings elsewhere were reported, and authors assert that even though the prevalence of rhesus negative was small in the population, the occurrence of a particular percentage no matter how negligible, may not be insignificant in view of the medical implications, mostly in the form of erythroblastosis fetalis that can lead to still birth.²⁵

Patients of blood group O+ recorded the highest parasite density (46.59% and 10.23%, representing mild and moderate parasitaemia respectively). Similar findings elsewhere revealed that blood group O seems to confer a certain degree of protection against severe courses of malaria disease.²⁶

The correlation analysis revealed a strong direct correlation between parasite density as it relates to blood group of patients examined. However, a significant correlation does not necessarily indicate causality but rather, a statistical linkage between levels of parasite density in a particular patient as it relates to his/her blood group type. The coefficient of determination (r^2) explains further the variability in parasite density as it relates to blood group of patients.

The coefficient of determination in this study, has shown that the variability in blood group accounted for 84% of the variability in parasite density. That is to say that differences in the ABO blood grouping system is responsible for the observed variation in parasite density. There is a general shortage of research in this regard and it is recommended that further studies need to be

conducted so as to unravel further, the relationship between genetic markers (with emphasis on the ABO-Blood group system) and malaria infection.

Based on the results of this study, it can be affirmed that mild/moderate courses of malaria parasite infection are highly prevalent among individuals with blood group O and that the density of parasites per microliter of blood is also more significant among patients with blood group O. Those with rhesus positive blood groups are more susceptible to infection than those that are rhesus negative and correlation coefficient between blood group and parasite density is strong, direct and linear.

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