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Prevalence of *Plasmodium Falciparum* among Medical Students with different ABO Groups and Electrophoretic Patterns in a Tertiary Institution in Nnewi, Nigeria

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Abstract
There is evidence that *Plasmodium falciparum (Pf)* malaria is influenced by ABO blood type but the extent of association is not fully established. Some investigators opinioned that haemoglobin electrophoretic patterns are a factor in susceptibility to *Pf* infection but there is no consensus on possible association between it and ABO blood group and Hb genotypes**.** This study was designed to determine the prevalence of *Pf* among different ABO blood groups and Hb electrophoretic patterns of medical students of a tertiary institution in Nnewi, Nigeria. A total of 80 subjects (41 males and 39 females) aged 18-30 years who reported to the Medical Centre of the institution on account of febrile illness were recruited for the study. Information on age, previous malaria episodes and recent use of prophylaxis were sought. Three milliliters (3ml) of blood were collected into EDTA container for ABO grouping, Hb electrophoresis and blood films for *P. falciparum* detection and quantification by microscopy. *Pf* prevalence among the subjects was 47.5% (38/80). Thirty-one (38.75%) of the subjects were of blood group O, 27 (33.75%) group A, 19 (23.75%) blood group B and 3(3.75%) blood group AB. Fifty-two (65%) of the subjects were Hb AA and 28 (35%) AS. No significance difference was seen between malaria episodes and ABO blood groups; Hb electrophoretic patterns; gender and parasite density ($p>0.05$) respectively. A negative correlation was observed between parasite density and age ($r= -0.180$, $p = 0.109$). *Pf* infection, frequency of infection and parasite load is not influenced by blood group and Hb electrophoretic patterns in our study population.

Keywords: *Plasmodium falciparum,* ABO Blood Groups, Hb electrophoretic patterns, Students, Nnewi.

Introduction

Malaria, a life-threatening parasitic disease caused by an obligate, intracellular protozoan parasite of the genus *Plasmodium.* The species infecting man include *Plasmodium falciparum, Plasmodium malariae, Plasmodium ovale, Plasmodium vivax* and *Plasmodium knowlesi.* While *P. vivax* is responsible for the largest number of malaria infections worldwide*, P. falciparum* accounts for about 90% of deaths from malaria (Mendis *et al.*, 2001). Africa had the highest burden of the disease, with 90% of cases and 91% deaths being from the region (WHO, 2017). In Nigeria, malaria one of the leading causes of mortality and morbidity, accounting for 15 % of all out-patient attendance in the country's health facilities (GOK, 2015). Two-thirds of all reported deaths were among children aged under 5 years (WHO, 2017). The World Health Organization (WHO) estimated that malaria causes 250 million cases of fever annually (WHO, 2009). In the year 2010 alone 655,000 people died from the disease (World Malaria Report, 2011). While another report put the death toll to 1,238,000 in same year 2010 (Murray *et al.*, 2012). Despite many multinational efforts towards its treatment and eradication, it has remained an important global public health disease. WHO estimate that 1.2 billion people to be at high risk of being infected and developing the disease, an estimate of 3.2 billion people in 95 countries and territories were at risk (WHO, 2016). Death toll had remained

around 445 000 annually (WHO, 2017). In an effort to control and eliminate this top killer disease, an estimated US$ 2.7 billion was spent in 2016 by international funding agencies with governments of endemic countries contributing just about 31%. Artemisinin-based combination therapies (ACTs) is currently the drugs of the first choice in the management of malaria, but concerns about the tastes of the oral preparation often affects compliance to prescribed regimens (Sonar *et al.,* 2017), apart from accessibility and affordability. WHO had suggested the inclusion of awareness and subsequent control of malaria in the school health curriculum (WHO, 2007). This, in their opinion, would assist in measures aimed at not only prevention but also the subsequent control of the disease.

Vector and host factors determine susceptibility to the disease. An example of vector factor is their feeding pattern which varies through the hours of a day, and even the seasons of a year. Some host factors such as the region of residence and lifestyles like personal hygiene that expose individuals to the vector and cooperation in control and eradication operations are modifiable (Ojiezeh *et al.*, 2010; William *et al.,* 2000). Hereditary and acquired factors are host-related factors that are not modifiable. Blood groups and Hb electrophoretic patterns are two examples of hereditary and host-related factors that have been established to be related with susceptibility to some diseases (Ito *et al.*, 2014).

There is increasing evidence that *P. falciparum* malaria is influenced by ABO blood type of an individual but the extent of association is not fully established (Ilozumba and Uzozie, 2009). Some investigators have expressed the opinion that Hb electrophoretic patterns is a factor in susceptibility to *Plasmodium* infection but there is lack of consensus on possible association between ABO blood group genes, genotypes and malaria parasitaemia. A number of studies have been conducted to investigate the association between ABO blood group system and some disease conditions (Tursen *et al.*, 2005; Blackwell *et al.,* 2002). Some of these studies reported significant associations, suggesting that ABO blood groups have an impact on infection status of the individuals possessing a particular ABO blood type (Abdulazeez *et al.*, 2008; Opera, 2007). Some studies however, reported the absence of significant association between *P. falciparum* and ABO antigens (Uneke *et al*., 2006). Variations in reports on the association of ABO blood groups, Hb electrophoretic patterns and disease progression of *P. falciparum* malaria show the complexity of the interaction between the parasites and host immune responses (Miller *et al.*, 1994). Understanding the nature of the relationship between ABO blood groups, genotypes, and *P. falciparum* infections would lead to development of control strategies with a definite target group within the population and hence reduce malaria transmission. Therefore, this study sought to investigate the relationship between ABO blood group phenotypes, Hb electrophoretic patterns and *P. falciparum* infections as a strategy towards understanding the host related factors that predispose and those that confer resistance to malaria infections.

It had been severally reported that several factors including developing immune response by the host, sickle cell trait, haemoglobin variants, ABO blood group and the level of G-6-P-D enzyme could modify susceptibility (Otajevwo, 2013). In view of a heavy burden placed on human health due to malaria, a good number of investigations have been conducted to find out whether or not ABO blood group antigens and Hb electrophoretic patterns are associated with susceptibility, resistance, or severity of *P. falciparum* malaria. Nonetheless, these studies have reported contradictory results and are therefore unclear. This is probably because the relations between the blood group and malaria have not been well studied especially in this locality. Therefore, an investigation into malaria in relation with ABO blood group has the potential of giving an insight into the pathogenesis of malaria and perhaps aid the control of this disease. The current study was designed to investigate the prevalence level of P. *falciparum* infections amongst individuals with different ABO blood groups and Hb electrophoretic patterns in relation to their age, gender and parasitaemia levels.

Materials and Methods

Study Design

The aim of this study was to assess the prevalence of *P. falciparum* malaria among

different blood groups and Hb electrophoretic patterns of medical students in a tertiary institution in Nnewi North Local Government Area of Anambra State, Nigeria.

Study Area

The study was carried out at a Medical College of a tertiary institution in Nnewi North Local Government Area of Anambra State, Nigeria whose coordinates is latitude 5.974 and longitude 6.892. The town experiences heavy rainfall and its environments are bushy with stagnant water. These favors plasmodium breeding and transmission. The study was carried out between the months of March to May 2021.

Study population

A total of 80 students aged 18-30 years who presented to the Medical Centre on account of febrile illness and gave their consents were recruited for the study. Only those who tested positive to *P. falciparum* specie were studied.

Ethical Approval

Ethical approval was sought and obtained from the institutional ethics committee (Reference No.: NAU/FHST/2021/MLS43) and written informed consent sought and obtained from the subjects.

Method of data collection/ Blood Sample Collection

Oral interview using English language was employed for data collection for age and previous malaria history and three milliliters (3mls) blood was drawn into EDTA-anticoagulated tube for blood smear, blood grouping and Hb electrophoresis.

Analytical Procedures

ABO and Rhesus D blood grouping was done using tube method along with standard cell as control, while Haemoglobin Electrophoresis was carried out by cellulose acetate paper at alkaline pH of 8.5 along with control samples. Identification and quantification of the parasites was done on 10% Giemsa-stained blood films (thick and thin) respectively. The number of parasites encountered among 100 WBCs were noted. A slide was considered negative if no parasite was encountered after examining 100 HPF and no malaria parasite was seen. The density was determined using the formula:

$$\text{Parasite Density } (\mu l) = \frac{\text{Number of parasites counted}}{\text{Number of WBC counted (100)}} \times 8000\mu L$$

Where; 8000 is the relative leucocytes per µl of blood (WHO, 1991).

Statistical analysis

Statistical analysis was done using statistical package for social sciences (SPSS), version 21. Results will be expressed as mean ±standard deviation. Kruskal-Wallis and chi square, was used for comparison between groups while Spearman's correlation and Mann Whitney was used to test for relationship and $p<0.05$ was considered statistically significant.

Results

Table 1 shows the prevalence level of *Plasmodium falciparum* parasitaemia among the study subjects. Table 2 shows the comparison of parasite density among different ABO blood groups. The parasite density (µl) of blood in descending order of blood groups were: blood group A (49.93±57.62); blood group O (34.58±53.40), blood group AB (32.00±55.43) and blood group B (20.21±32.37). But the different was not statistically significant ($p>0.05$). Table 3 compares the parasite density among subjects with Hb electrophoretic patterns AS and AA. The result pattern shows that parasite density is higher in subjects who were AS (40.71±51.69) compared to those who were AA (33.85±51.25) ($p>0.05$). Table 4 shows the correlation between parasite density and age. The result shows a negative correlation with no significant difference ($p>0.05$). Table 5 shows the comparison of malaria episodes among different ABO blood groups. The percentage malaria episode was in this order; blood group O (38.8%), A (33.8%), B (23.8%) and AB (3.8%). ($p>0.05$). Table 6 compares the Hb electrophoretic patterns with malaria episodes. There was no significant difference between Hb electrophoretic patterns and malaria episodes ($p>0.05$) although, malaria episode was more in subjects who were AA (65.0%) compared to those who were AS (35.0%). Table 7 compared gender with malaria episodes. There was no statistically significant difference between gender ($p>0.05$). Female had (48.8%) while male (51.2%).

Table 1: Prevalence of *Plasmodium falciparum* parasitaemia among the subjects

Parameters	Number	Percentage
Presence of parasites	38	47.5%
Absence of parasites	42	52.5%
Total	80	100%

Table 2 Level of parasite density among different blood groups

Group	Parasite density	Kruskal-Wallis	p-value
O	34.58±53.40		
A	49.93±57.62	2.383	0.497
B	20.21±32.37		
AB	32.00±55.43		

Table 3 Level of parasite density in AS and AA genotype

Parameter	AS	AA	Mann-Whitney U	p-value
Parasite density(µl)	40.71±51.69	33.85±51.25	752.00	0.620

Table 4: Correlation of age with parasite density using Spearman

Parameter	R	p-value
Age(years) vs parasite density(µl)	-0.180	0.109*

*p<0.05= significant

Table 5: Comparison of blood groups with malaria episode

	Blood group	Weak	Mild	Heavy	Total	Chi square	p-value
	Group O	23	3	5	31		
		39.7%	25.0%	50.0%	38.8%		
	Group A	16	7	4	27		
		27.6%	58.3%	40.0%	33.8%		
	Group B	17	1	1	19	7.655	0.264
		29.3%	8.3%	10.0%	23.8%		
	Group AB	2	1	0	3		
		3.4%	8.3%	0.0%	3.8%		
	Total	58	12	10	80		
		100.0%	100.0%	100.0%	100.0%		

Table 6: Comparison of HB Electrophoretic Pattern with malaria episode

HB Electrophoretic Pattern	Weak	Mild	Heavy	Total	Chi square	p-value
AS	19	6	3	28		
	32.8%	50.0%	30.0%	35.0%		
AA	39	6	7	52	1.425	0.490
	67.2%	50.0%	70.0%	65.0%		
Total	58	12	10	80		
	100.0%	100.0 %	100.0%	100.0%		

Table 7: Comparison of gender with malaria episode

Gender	Weak	Mild	Heavy	Total	Chi square	p-value
Female	29	5	5	39		
	50.0%	41.7%	50.0%	48.8%	0.285	0.868
Male	29	7	5	41		
	50.0%	58.3%	50.0%	51.2%		
Total	58	12	10	80		
	100.0%	100.0%	100.0%	100.0%		

Discussion

The prevalence of *Plasmodium falciparum* specie was studied with the aim of determining possible association between it and ABO blood group and HB Electrophoretic Pattern among students of College of Health Sciences of a tertiary Institution in Nnewi. We report that almost half of the student who presented with febrile illness to the Medical Centre had *P. falciparum* and that blood groups and HB Electrophoretic Pattern had no influence on parasite density ($p>0.05$). This present finding is consistent with previous reports (Omotade *et al*., 1999; Ademowo *et al*., 1995) who equally reported no association between ABO blood group and malaria parasitaemia. These genes were also seen not to modify susceptibility in consonant with the finding of Otajevwo *et al*. (2013). However, our result pattern contrasted that of Nkuo-Akenji *et al.* (2004) which rather reported that blood group O individuals were more susceptible to malaria attack than other ABO groups. Our result favors the null hypothesis which states that there is no association between ABO blood groups and *P. falciparum* parasitaemia. Again, we could not establish an association between parasite density and the genetic variables. The improved healthy lifestyle of the students such as cleanliness, improved feeding and dress code of the students (most students of all gender now wear trousers which prevents exposure and mosquito bites) reduces malaria replication and consequently the parasite load. Even though we did not consider the immune status of our study subjects, the level of immunity of these young subjects who are at the prime of their life could have been a strong factor in favour of non-susceptibility. The result of this study also suggests that the ABO blood group of an individual does not determine the level and severity of malaria symptoms expressed. It had earlier been documented that not all individuals with malaria parasitaemia have clinical symptoms and parasite density had

no correlation with severity (Newton *et al.,* 1997). We equally report that blood group and Hb electrophoretic pattern does not have association with the frequency of malaria (episodes) and also gender has no association with malaria infectivity as earlier documented by Sirina and Okpoku-Okrah (2013) who posited that males had a significantly higher infection rate and symptoms than females.

Conclusion*:* *Pf* infection, frequency of infection and parasite load is not influenced by blood group and haemoglobin electrophoretic pattern of our study population.

Recommendations: It is recommended that the sample size be increased in future study.

Conflict of Interest: The authors declare no conflict of interest.

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