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Prevalence of Malaria Parasitaemia and Its Association with ABO Blood Group in Jos, Nigeria

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Abstract: Malaria is a disease associated with high morbidity and mortality especially among children and other high risk groups. Certain ABO blood group is thought to be protective of severe malaria. Determining the prevalence of malaria parasitaemia among asymptomatic individuals and its association with ABO blood grouping could be a step to further studies to understand the immunity of Malaria. A cross sectional study in which one hundred and seven asymptomatic, consenting individuals in malaria endemic Jos, Nigeria, were recruited into the study. Demographic data and blood samples were taken for the determination of ABO blood group and for thin and thick blood film for malaria parasite detection. The asexual parasite density (asp/µl) was also determined. Of the 107 individuals studied, there were 30(28.0%) males and 77(72.0%) females. The mean age of study participants was 26.2±6.3 years. The predominant blood group was O 48(44.9%), followed by B 33(30.8%), A 21(19.6%) and AB 5(4.7%) respectively. The prevalence of Malaria parasitaemia was 40(37.4%). The mean parasite density was 241.0± 69 asp/µl. There was a significant association between Malaria parasitaemia and ABO blood group (P<0.017). The prevalence among the same blood group was: A 12(57.1%); O 21(48.8%); AB 1(20.0%) and B 6(18.2%) respectively. Across the ABO blood groups, females were significantly parasitaemic as compared to males (*P*> 0.016, 0.03 and 0.026 respectively) The median WBC count of Malaria infected individuals was significantly higher than the median WBC count of those subjects without parasitaemia, (P<0.01). There was a positive correlation between the total White blood cell count (WBC) count and asexual parasite density, although only 35.4% was attributable to the WBC (r^2=0.354, *P*<0.0001). In conclusion, the prevalence of Malaria parasitaemia in our cohort is comparatively low to southern parts of Nigeria. Although there was relative spread of parasitaemia across all blood groups, the highest rate was observed among blood group A and in females. We recommend that available malaria interventions should be directed at all individuals but with particular emphasis on Blood group A and females. Large studies are required to validate our findings, and to elucidate the socio-demographic and immunologic mechanisms involved in the apparent protection.

Keywords: Malaria, Parasitaemia, ABO Blood Group, Jos, Nigeria

1. Introduction

Malaria is a disease caused by *Plasmodium* species which is transmitted by the female anopheles mosquitoes. In 2015, 429,000 people died from Malaria globally. The WHO African Region accounted for 90% global cases of Malaria and 92% global mortality, where *Plasmodium falciparum* is the most prevalent of the Malaria parasites [1].

It has been observed that despite the high morbidity and mortality, certain individuals living in Malaria endemic regions appear relatively protected from frequent severe malaria attack. This resistance to Malaria is dependent on the development of a protective immune response by the host [2]. The ABO blood type is thought to play an important role

in the protection against severe malaria [3]. The *ABO* blood group gene has three alleles namely; A, B and O. It codes for different types of agglutinogens attached to the surface of red blood cells (RBCs) and hence determining an individual's blood group. The plasmodium parasite has established a close relationship between itself and the RBCs. The severe pathophysiological manifestations of Malaria, caused by *Plasmodium falciparum* are a direct consequence of the parasite's blood stage replication cycle, during which merozoites repeatedly invade, multiply within, and destroy RBCs. Consequently, RBCs have evolved specific receptor-ligand interactions, some of which involve the ABO blood group antigens, to facilitate their adherence and invasion by merozoites. Therefore, any variation in the erythrocyte ABO antigens can change the penetration and establishment of the parasite in the RBCs [4]. There are epidemiological evidence that the ABO phenotype may modulate disease severity and outcome of *P. falciparum* malaria.

Clinical studies conducted in Thailand and East Africa demonstrated that the frequency of resetting parasites in blood isolated from group O patients was less than in blood isolated from patients with blood groups A, B and AB [5]. Other studies have also reported that *P. falciparum* forms rosettes with group O RBCs with lesser frequency compared with group A and B [6]. In addition, the rosettes formed with group O RBCs have been reported to be smaller and more easily disrupted than those formed in groups A, B and AB erythrocytes. The plasmodium parasite has been observed to have a reduced capacity to invade group O erythrocytes [7] while macrophages targeting *P. falciparum* infected erythrocytes have been shown to clear infected O erythrocytes more avidly than infected A and B erythrocytes [8]. This could indicate some resistance of group O to the severe presentation of malaria. This association is consistent with the observed predominance of blood group O in malaria endemic sub-Saharan Africa relative to other parts of the world where malaria is not endemic [9]

A high percentage of severe malaria cases have been reported among blood group A individuals [10, 11]. Furthermore, several studies had demonstrated low parasitaemia and uncomplicated malaria cases among blood group O individuals [12-16]. Beigulman *et al* and Akhigbe *et al* reported that blood group AB persons had the lowest malaria attack while blood group A persons had the highest attack [17, 18].

Studies to investigate any possible association between ABO blood group system and some disease conditions have been carried out by some authors [19-22]. Some of these studies reported significant associations thereby suggesting that ABO blood groups have an impact on the infection status of the individuals possessing a particular ABO blood group [20-22]. The absence of significant association between *P. falciparum* prevalence and ABO antigens has also been reported by some other studies [6, 15, 23].

Studies in Nigeria have also demonstrated some association between malaria parasitaemia with ABO blood types. In Warri, the highest malaria parasite load was observed among group O (52.2%) individuals while the least was noticed among blood group AB (8.7%) individuals. Malaria parasitaemia was higher among the males (83.3%) than females (75.0%) [24]. In another study in Edo State University, blood groups O and B male individuals were the most and least susceptible to malaria attack respectively [3].

There is still no consensus on association between ABO blood group and Malaria infection [6] Therefore an understanding of the nature of relationship (if any) between ABO blood groups and malaria parasitaemia should provide an invaluable window in the scourge and studies of malaria parasitaemia from that stand point in populations of Malaria endemic regions will be helpful in elucidating any such relationship [25]. Therefore, there is the need to conduct this study to provide a preliminary comparative description of the prevalence of malaria parasitaemia and its association with ABO blood grouping.

2. Materials and Methods

2.1. Study Area and Design

This descriptive study was carried out at the Plateau State Specialist Hospital Jos, in Jos metropolis which has a population of about 900,000 inhabitants [26]. The climatic conditions are typical of a tropical region which may aid malaria transmission. One hundred and seven asymptomatic individuals were randomly selected for the study. Informed oral and written consent was obtained from the participants. Data collected includes age, sex, educational status and use of insecticide treated net (ITN). 5ml of blood sample was also collected through venepuncture from the participants according to standard procedure for determining the ABO blood group and blood film was prepared for parasite detection and estimation of parasite density.

2.2. Typing Blood Samples for ABO Blood Group Antigens

The ABO blood group of each subject was determined using cell grouping Antisera according to methods described by Rosenfield and Cheesbrough [27, 28]. Monoclonal Antisera A, B and D (Agappe Diagnostics Ltd, India) were used.

2.3. Blood Film Parasitological Examination

The diagnosis of malaria was done by detecting and identifying malaria parasites in blood films using light microscopy at x100 magnification. Thick blood film was used to determine the parasite densities while thin blood films was used for species differentiation (confirm Plasmodium species) if doubtful on thick films. The asexual parasite density (asp/ μL) was estimated using the formula = (Number of parasites counted/WBC counted) × WBC count/ μL of blood as previously described by O'Meara *et al* [29].

2.4. Ethical Consideration

Ethical clearance for the study was obtained from the

Ethical Review Committee of the Plateau State Specialist Hospital, Jos. Informed written and oral consent was also obtained from the patients.

2.5. Statistical Analysis of Data

Data was entered into Microsoft Excel and analysed with Statistical Package for Social Sciences (SPSS Version 17, Chicago, IL). Categorical variables were analysed for associations using the chi-square test while continuous variables were analysed using Students t-test for significance. Logistic regression was also done to determine independent associations. *P*-values <0.05 was considered as significant.

3. Results

One hundred and seven individuals were included in the study. There were 30(28.0%) males and 77(72.0%) females. The mean age of study participants was 26.2±6.3 years; they were predominantly less than 35 years; 93(86.9%). The predominant blood group was O 48(44.9%) followed by B 33(30.8%), A 21(19.6%) and AB 5(4.7%) respectively. The prevalence of Rhesus negative was 33(30.8%), with 9(8.7%) males and 33(30.8%) females.

The prevalence of malaria parasitaemia was 40(37.4%). The mean parasite density was 241.0 ± 69 asp/µl. There was however no significant association between age, sex and having malaria parasitaemia, (P=0. 26, P=0.59 respectively). Although our study did not show an age-related association to malaria parasitaemia, there was a trend for malaria to be associated with younger adults <35 years than in older adults ≥35 years, *(P*=0.055). There was similarly no association between educational status and ITN usage (Table 1).

There was however a significant association between malaria infection and ABO blood groupings (P<0.017). The prevalence among the same blood group was: A 12(57.1%); O 21(48.8%); AB 1(20.0%) and B 6(18.2%) respectively (Table 1).

Age, sex, and ABO blood group were not significant as independent predictors of parasite density (Table 2)

Among the different ABO blood groups (A, B and O) females have significant parasitaemia (P> 0.016, 0.03 and 0.026 respectively) Table 3.

The median WBC count of subjects who have Malaria parasitaemia was significantly higher than the median WBC count of those subjects who did not have Malaria parasites, $6.3x10^3$/µl vs. $5.6x10^3$ / µl, P<0.01.

We observed a positive correlation between the total WBC count and parasite density, r^2=0.354, *P*<0.0001 (Figure 1).

Table 1. *Demographic Characteristics of study participants.*

		Malaria Parasites Test		
Variables	**Total (n=107)**	**Positive (n=40)**	**Negative (n=67)**	***P*-Value**
Age (Years)				
Mean Age	26.2±6.3	25.6±5.1	26.9±6.9	0.26
<35 Age group	93(86.9)	38(95.0)	55(82.1)	0.06
≥ 35 Age group	14(13.1)	2(5.0)	12(17.9)	
Sex				0.59
Male	30(28.0)	10(25.0)	20(29.9)	
Female	77(72.0)	30(75.0)	47(70.1)	
Education Status				0.93
No Formal	7(6.5)	2(5.0)	5(7.5)	
Primary	3(2.8)	1(2.5)	2(3.0)	
Secondary	9(8.4)	4(10.0)	5(7.5)	
Tertiary	52(48.6)	20(50.0)	32(47.8)	
Missing Values	36(33.6)	13(32.5)	23(34.3)	
ITN Usage*				0.07
Yes	5(4.7)	0(0.0)	5(7.5)	
No	102(95.3)	40(100.0)	62(92.5)	
Blood Group				0.017
A	21(19.6)	12(30.0)	9(13.4)	
B	33(30.8)	6(15.0)	27(40.3)	
AB	5(4.7)	1(2.5)	4(6.0)	
O	48(44.9)	21(52.5)	27(40.3)	
WBC Count X10^3/l Median (IQR)§				
Total	6.3(5.0-7.4)	6.3(5.5-8.2)	5.6(4.8-7.0)	0.01
Male	5.6(4.98-6.8)	6.5(5.5-7.3)	5.3(4.9-6.58)	
Female	6.3(5.1-7.4)	6.3(5.47-8.2)	6.3(4.8-7.4)	

*Insecticide Treated Net

§ White blood Cell Count

Table 2. *Multivariate analysis for independent predictors of asexual parasite density.*

			Logistic Regression
Variable	**Mean (ASP/µl)**	**Adjusted aOR (95%CI)**	***p*-value**
Age			0.69
<35 years	241.1±71.1	2.6(0.5-14.4)	
>35 years	238.5±27.6		
Sex			0.48
Male	240.0±73.4	1.1(0.4-3.2)	
Female	241.2±69.4		
ABO blood group			0.47
A	244.3±85.6	8.6(0.7-110.1)	
B	228.8±30.2	1.4(0.1-17.6)	
AB	400		
O	235.2±61.5	5.5(0.5-64.9)	

Table 3. *Asexual parasite (ASP/µl) distribution by sex.*

	Male	**Female**	***p*-Value**
Mean	240.0±73.4	241.2±69.4	0.147
ABO Grouping			
A	287.6±94.8	222.7±77.8	0.016
B	228.3±45.4	228.3±15.3	0.03
AB	0	400	
O	189.5±9.9	242.8±63.3	0.026

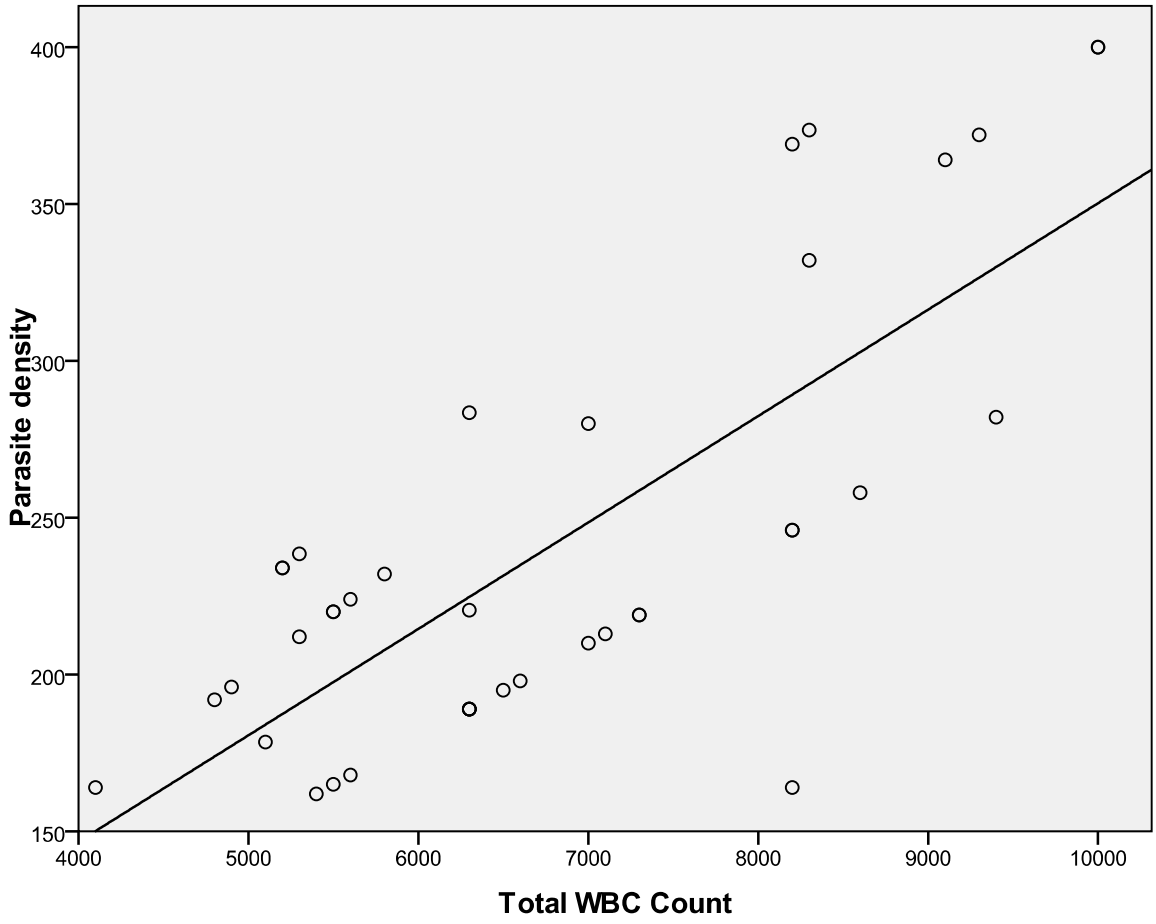

Figure 1. *Relationship between parasite density and total WBC count.*

4. Discussion

Malaria is an important public health concern due to its associated high mortality and morbidity in many parts of the world. It is therefore, important to identify the factors which contribute to susceptibility of hosts. Results obtained in this study showed that of the 107 sampled individuals made up of 30(28.0%) male and 77(71.9%) female patients, 5(4.7%), 21(19.6%), 33(30.8%) and 48(44.9%) belonged to blood groups AB, A, B and O respectively in that increasing order. These findings showed that the most prevalent blood group is O 48(44.9%) and the least, AB 5(4.7%). This is consistent with most report in Malaria endemic zones showing a predominance of blood group O and AB being the least [9, 24, 25, 30]. Thought there are variations in the figures but the pattern is maintained in all the studies.

In this study there were 9(8.7%) and 24(22.4%) male and female rhesus negative individuals respectively. Although the prevalence of rhesus negative factor in the population is small, the occurrence of 33(30.8%) out of a sample population of 107 may not be insignificant in view of the medical implications arising from hemolytic disease of the newborn.

The study found a prevalence of Malaria parasitaemia of 40(37.4%). This parasitaemia rate appears to be low when compared to 76.8% and 93.4% obtain in Okada and Odoakpu respectively [25, 31]. Parasitaemia rate varies with location as shown in different studies in Nigeria; 79.3%, 77.4%, 58.3%, 43.2%, 10.0% and 6.0% obtained respectively from blood donors in Warri [24], blood donors in Owerri [32], Children in Awka [33], blood donors in Ibadan [34], coastal dwellers of Lagos State [35] and blood donors in Maiduguri [36]. These results may suggest existence of regional differences in Malaria parasitaemia in Nigeria, with the mid-western and eastern areas (represented by Warri South LGA, Onitsha South LGA and Owerri) ranking highest in prevalence rating and the northern area (represented by Maiduguri) occupying the lowest position, while the Western area (represented by Lagos State and Ibadan takes a middle position. The high prevalence rate in Warri may be due to the swampy nature of the terrain as well as the densely populated nature of the area owing to its oil rich disposition which is in contrast with the north that is mainly savannah vegetation and less dense population [24]. Results of more studies on malaria parasitaemia in different parts of the country would be needed before a more definite statement on the apparent trend could be made. The somewhat low malaria parasitaemia rate recorded among our cohort may be as a result of the improved sanitary conditions, the use of intermittent malaria prophylaxis as well as geographic factors.

Among male individuals screened 10(33.3%) had Malaria parasitaemia compared to 30(38.9%) female individuals with parasitaemia. Although this was not statistically significant (P<0.59), it shows a higher infection rate in female patients in relation to their male counterparts. This report is not consistent with trends observed in Warri, Ekpoma, Odoakpu, Owerri and the coastal areas of Lagos [3, 24, 25, 32, 35, 37]. The reasons for the observed sex differences are not far-fetched as some of the females may be pregnant which may increase their predisposition to Malaria, more studies among pregnant women and other populations in other parts of Nigeria would be expedient to offer possible explanation.

The study also showed that the highest Malaria parasitaemia rate was observed among the A blood group with 12(57.1%). This was followed by blood group O 21(48.8%), blood group AB 1(20.0%) and blood group B 6(18.2%) respectively. Studies in Warri and Odoaku found 100.0% malaria parasitaemia among blood group AB individuals followed by 94.9% among blood group O individuals and this is not consistent with findings of the present study [24, 25]. Findings in this study also disagree with earlier reports [38, 39], who observed low malaria parasitaemia among blood group O individuals. In their reports, they concluded that blood group O seems to confer a certain degree of protection against severe Malaria. It is noteworthy that blood group A subjects recorded the highest parasitaemia rate but it seems there is relative spread of malaria parasitaemia across all blood groups in this study. This finding is similar with a study by Fischer and Boone of which they reported that malaria occurs in patients of any blood group and that no particular blood group precludes the possibility of severe malaria [10]. In another study in Ghana among blood donors there was no significant difference in parasitaemia among the different blood groups [40]. These varied findings may be due to some biological and geographical factors depending on the location.

Females constituted the majority in our cohort, but there was no statistical significant difference in terms of parsitaemia between males and females (P>0.147), but among the different ABO blood groups (A, B and O), females have significantly higher parasitaemia when compared to males in the same ABO group. This does not tally with the suggestion of Portilo and Sullivan that genetic factors could play a role by endowing females with immuno-regulatory potentials to cope better with some diseases [41]. Again, the occurrence of a higher malaria parasitaemia rate among the female participants may be a matter of chance; a larger sample size could give a better picture.

In our cohort there was a relatively low total WBC count and individuals with parasitaemia have a significant higher total WBC count. There was a positive correlation between total WBC and parasite density. Only 35.4% of variations in parasite density could be attributed to changes in total WBC, hence the use of the assumed value of 8,000 cells/μL to estimate parasite density could lead to an overestimation [42]. A previous study carried out in Nigeria, reported that parasite density was overestimated using this method in children infected with *P. falciparum* [43]. Another study conducted in 3,044 African children with acute malaria showed that the discrepancy in the estimated parasite density using an assumed WBC count of 8,000 cells/μL was higher in younger children. In older children, a greater similarity with the automated WBC estimate was observed [44].

Therefore in this study absolute total WBC count was used to overcome overestimation of the parasite density.

Although this is the first study of its kind in our locality the small sample size can limit the generalization of the findings, a study with a larger sample size could give a better outlook.

5. Conclusion

The prevalence of Malaria parasitaemia in our study is comparatively low to the southern part of Nigeria. There was a statistical significant association of malaria parasitaemia and ABO blood group. Blood group A and females have higher rates of parasitaemia, although there was a relative spread of parasitaemia across all blood groups. We recommend that available malaria prophylactic strategies should be directed at individuals of all blood groups with particular emphasis on Blood group A and females. Further studies would be required to validate our findings and also elucidate on the socio-demographic and immunologic mechanism in the apparent protection conferred on males and the other blood groups.

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