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Malaria parasitemia and its association with ABO blood group in Imo State, Nigeria

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Abstract
Malaria parasitemia and its severity have been associated with many host attributes including ABO blood groups. Using cross-sectional study design, six hundred and twenty five (625) respondents were randomly selected from three senatorial districts of Imo State. Structured questionnaire and laboratory diagnosis were the tools used in eliciting the information needed for the study. Data were subjected to descriptive statistics, analysis of variance (ANOVA) and Chi-square using SPSS statistics version 23. The respondents were dominated by male 331(53%) youths who were within the age range of 31-40 years 152(24%) and were mostly residing in the urban area 100(16%). They had primary school education 219(35%) as their highest level of education. Almost half of them belong to O blood group 296(47%). Laboratory diagnosis showed that out of the 625 participants, malaria parasitemia was high in 395(64%) respondents especially in the rural area 203(32.5%). Besides, Owerri zone 173(28%) had more malaria subjects than other senatorial zones with significant association ($p = 0.018$). *Plasmodium faciparium* was the major parasitemia suffered by the subjects 358(90%). Most of the subjects 246(62%) had low malaria parasitemia. Blood group had significant influence on malaria parasitemia such that participants belonging to O blood group 187(47%) had more malaria. Efforts should be made by government in improving access to health care in malaria endemic states as this will help to reduce the malaria parasitemia.

Keywords: blood group, malaria, parasite, rural area

Introduction

Malaria disease is the highest widespread tropical disease characterized with high morbidity and mortality and subsequent social and economic impacts and as such continue to be a principal public health problem in mostly Nigeria rural populations where poor housing, improper sanitation and stagnant water predisposes to malaria disease, as is the same elsewhere in Africa [1, 2]. It contributes 30% of in-patient admissions, about 50% of out-patient visits and 40% of public health expenditure in areas with high malaria prevalence [3, 4, 5]. At least fifty percent of Nigeria population suffers from at least one incident of malaria every year [6]. Five *Plasmodium* species: *Plasmodium vivax, P. malariae, P. falciparum, P. ovale* and *P. knowlesi* cause malaria in human, each with their variable incubation periods and geographical location with 80% of infections and 90% of deaths globally caused by *P. falciparum* [7, 8] and hence continues to possess an obvious impact on public health in the tropics [9].

After the bites of infectious *Anopheles gambiae (*mosquitoe), about 3000 sporozoites is introduced into the person at every feeding [7]. These sporozoites travel to the liver where many developments take place [10]. According to [11], vulnerability to malaria infection differs with location and individuals [9]. Argued that a good knowledge of the factors related with the immunity to and protection from malaria disease may assist in the production of its vaccines and other therapies. Susceptibility of the host to malaria infection and disease is controlled by hereditary and acquired factors like genotypes (AA, AS, and SS) and human blood groups (ABO) [12]. Blood group antigens are determined by hereditary factors and it plays a crucial role in understanding genetics, inheritance pattern, transfusion safety, and disease susceptibility [12, 13].

Some researchers have demonstrated that ABO blood groups have an effect on infection status of a person [14, 15]. It was stated by [16] that RBCs infected by malaria often bind to uninfected RBCs to produce clumps called rosettes. These rosettes have the capability of obstructing flow in small blood vessel leading to damage of tissue and serious malaria infection. The virulence of *Plasmodium falciparum* has been linked to the ability of the infected RBCs to be held to uninfected RBCs and this leads to rosetting of cells [17, 18]. Infected patients can develop a varying range of one or more symptoms that decide one of the above clinical stages of malaria: severe, mild or asymptomatic malaria. ABO blood groups are made up of A, B and H carbohydrate antigens that can control the activities of protein during infection stages and antibodies against these antigens [19, 20]. According to [21], so many relationships have been observed between the ABO blood group pattern and some disease conditions including onchocerciasis, schistosomiasis, cancer, and HIV infection.

Malaria and ABO blood group have both been studied for over 100 years, and there are numerous articles on impact of ABO blood group on different kinds of malaria from so many countries. Many studies were carried out to evaluate the relationship between ABO blood group system and some human diseases including malaria. According to [22], some of the findings reported strong associations, indicating the effect of ABO blood system on infection status of an individual with a peculiar ABO blood system. People with blood group A and B easily contacts malaria infection when compared with people with O blood group. However,

infection severity varies due to varying host susceptibility [23, 24]. Moreover, many other findings reported absence of serious relationship of malaria with all ABO blood groups system, indicating that all persons with any kind of blood group are being equally vulnerable to malaria infection [25, 26, 11]. [27] Suggested that the controversial relationship of ABO blood groups system and severe malaria is closely determined by to the geographic distribution of the various blood groups systems within the endemic zones. These studies have been conducted in various tropical countries affected by malaria around the globe, however, in Imo State, Nigeria, a State with large endemic zones; there are few similar studies or statistic evidence about this alleged relationship as well as the hematological variables. Therefore, this study determined the association between malaria parasitemia and blood group in Imo State.

Materials and Method

Area of Study

The study area is Imo State which is one of the 36 states of Nigeria and it is in the southeast region of the country. Owerri is its capital and among the largest cities in the state. Other major cities are Obowo, Orlu, Mbaise, Oguta, and Okigwe. It occupies the area between the lower River Niger and the middle and upper Imo River. The estimated population is 4.8 million and the population density varies from 230-1400 people per sq km (NPC, 2010). The area lies within latitudes $4^0 45^{'}$N and $7^0 15^{'}$N, and longitude $6^0 50^{'}$E and $7^0 25^{'}$E with an area of around 5,100 sq km. The state is divided into three senatorial zones/districts namely Imo east (Owerri), Imo west (Orlu) and Imo north (Okigwe) senatorial zones. The rainy season begins in April and lasts until October with annual rainfall varying between 1500mm and 2,200mm (60 to 80 inches). An average annual temperature above 20^0c (68^0F) creates an annual relative humidity of 75% with humidity attaining 90% in the rainy season. The dry season experiences two months of harmattan from late December to late February.

Study Design

The study design used in this study is a comparative study design and a cross-sectional study design. The study was carried out between March 2020 to December 2020 to determine the association between malaria parasitemia and blood group in Imo State.

Study Population/Sample Size

The population of this study comprise all age groups of both sexes residing in both urban and rural areas of Imo State. The estimated total population of persons in Imo State is 1,1427,80 according to 2019 projected population estimation. The LGA of interest include Owerri North, Aboh Mbaise, Oru-East, Oguta and Ehime Mbano with an estimated 2019 population size of 264,846, 295,435, 168,851, 215,942 and 197,706 respectively given a total population of 1,142,780. The sample size was obtained from the population of the study using [28] formular.
This is given as

$$n = N/1 + N(e)2$$

n = Sample size
N = Population
1 = Constant
e = Degree of error expected

Where
N = 1142780
e = 0.05

Therefore n is given as

$$n = \frac{1142780}{1 + 1142780(0.05)^2}$$

$$n = \frac{1142780}{1 + 1142780(0.0025)}$$

$$n = \frac{1142780}{1 + 2856.95}$$

$$n = \frac{1142780}{2857.95}$$

n = 400

Attrition rate/loss rate/over sampling

56.2%= 56.2/100*400= 224.8=225
225+400= 625
625 becomes the new sample size

Sampling Technique

Multi-stage systematic cluster sampling techniques was used for the study in which three senatorial zones of Imo State were chosen, thereafter, three rural and three urban areas were selected from which one community each was randomly selected. On arrival at the selected villages, the appropriate consent was sorted from the community. At the middle of the village a bottle was spined and allowed to settle, all the households to which the mouth of the bottle points were sampled. Questionnaire was administered to collect some socio demographic data.

Specimen Collection/Laboratory Procedures

5 ml of blood was collected by the Laboratory scientist into ethylene-diamine-tetra-acetic acid (EDTA) anticoagulated blood container tubes. This was centrifuged at 3500 rpm for 10 minutes to disintegrate the red blood cells from the plasma, buffy coat and was stored at ~80 ºC for later analysis.

ABO Blood Grouping

The ABO blood typing of each respondent was determined using cell grouping Antisera as described by [29]. Monoclonal Antisera A, B and D (Agappe Diagnostics Ltd, India) were used.

Blood Film Parasitological Examination

Malaria infection diagnosis was conducted by detecting and identifying malaria parasites in blood films using light microscopy (at x100 magnification). Parasite densities was determined with thick blood film whereas species differentiation (confirm Plasmodium species) was determined with thin blood films if doubtful on thick films. Asexual parasite density (asp/ µL) was determined using the formula described by [30]; (Number of parasites counted/WBC counted) × WBC count/ µL of blood.

Ethical Consideration and Informed Consent

Approval to conduct the study was sought before the

commencement of the study from Ethical and Research Review Committee, Imo State University Owerri. Written informed consent was administered to the potential subjects and only those who gave their consents were recruited into the study. Interviews were conducted in such a manner that ensured confidentiality and privacy of the subjects.

Method of Data Analysis

Data analysis was conducted using IBM-SPSS statistics version 23 (SPSS Inc. Chicago, USA) for data analyses. Chi-square was used to ascertain the difference between frequencies and the relationship between blood groups and malaria parasitemia. Analysis of Variance at 5% probability level was used to estimate the difference between means.

Results

Demographic Characteristics of the Respondents Studied

Results of Table 1 displayed the demographic characteristics of the respondents studied. It was shown that the age range of the respondents varied significantly (P< 0.05). Age bracket of 31-40 years dominated the urban area of Orlu 31(40%), Owerri 40(33%) and Okigwe 29(33%) whereas; 51-60 years age group dominated the rural area of Orlu 17(22%), Owerri 33(24%) and Okigwe 22(24%). Categorically, the respondents in urban area 100(16%) and rural area were in the age brackets of 31-40 years and 51-60 years respectively (fig 1a). Majority of the participants in Orlu 72(71%) and Owerri urban areas were male whereas majority 54(58%) of them in Okigwe area were female. Similarly, in the rural areas of Orlu 44(56%) and Okigwe 62(67%) were female compared to more 68(50%) male respondents recorded in Owerri rural area. Generally, it was shown that the majority of the respondents were male 197(32%) and female 174(28%) in urban and rural settlements respectively.

In Table 1, it was shown that greater proportion of the subjects in urban areas of Owerri 50(41%) and Okigwe 39(42%) including rural area of Okigwe 36(39%) unlike greater proportions 44(%) that were in secondary school in Orlu urban area. In the rural areas of Orlu 29(37%) and Owerri 61(45%) had no formal education. Overall, most of the respondents studied were in primary school 109(17%) and had no formal education 21(121%) in urban and rural area respectively.

Majority 296(47) of the participants were in O blood group. It was shown that majority of the participants in urban 163(26%) and rural area 133(21%) belonged to the O blood group compared to other blood groups with significant association.

Table 1: Demographic characteristics of the respondents studied

Frequency (%)								
Variable	**U1**	**U2**	**U3**	**R1**	**R2**	**R3**	**Total**	**ANOVA**
Age range (Years)								
0-14	8(8)	14(11)	7(11)	5(6)	15(11)	14(15)	63(10)	10.5
15-30	23(23)	33(27)	20(27)	14(18)	21(15)	12(13)	123(20)	20.5
31-40	31(31)	40(33)	29(33)	15(19)	21(15)	16(17)	152(24)	25.3
41-50	17(17)	19(15)	17(15)	15(19)	25(18)	15(16)	108(17)	18
51-60	13(13)	11(9)	9(9)	17(22)	33(24)	22(24)	105(17)	17.5
61 or older	9(9)	6(5)	11(5)	13(16)	22(16)	13(14)	74(12)	12.3
Total	101(100)	123(100)	93(100)	79(100)	137(100)	92(100)	625(100)	LSD=5.81**
St.D	8.86	13.28	8.24	4.22	5.95	3.56		
X^2: 53.07; p < 0.001								
Gender								
Male	72(71)	86(70)	39(42)	35(44)	69(50)	30(33)	331(53)	55
Female	29(29)	37(30)	54(58)	44(56)	68(50)	62(67)	294(47)	49
Total	101(100)	123(100)	93(100)	79(100)	137(100)	92(100)	625(100)	LSD= 33.6**
St.D	30.4	34.6	10.6	6.4	0.7	22.6		
X^2: 50.4; p < 0.001								
Highest level of education								
No formal education	10(10)	6(5)	5(5)	29(37)	61(45)	31(34)	142(23)	24
Primary school	20(20)	50(41)	39(42)	26(33)	48(35)	36(39)	219(35)	37
Secondary school	44(44)	28(23)	11(12)	19(24)	15(11)	18(20)	135(22)	23
Tertiary school	22(22)	29(24)	31(33)	4(5)	10(7)	7(8)	103(16)	17
Post graduate	5(5)	10(8)	7(8)	1(1)	3(2)	0(0)	26(4)	4
Total	101(100)	123(100)	93(100)	79(100)	137(100)	92(100)	625(100)	LSD= 16.13
St.D	15	17.6	15.4	12.7	25.5	15.3		
X^2: 174.35; p < 0.001								
Blood group								
0	61(60)	60(49)	42(45)	37(47)	65(47)	31(34)	296(47)	49
A	30(30)	48(39)	36(39)	24(30)	50(36)	28(30)	216(35)	36
B	7(7)	10(8)	9(10)	13(16)	16(12)	25(27)	80(13)	13
AB	3(3)	5(4)	6(6)	5(6)	6(4)	8(9)	33(5)	6
Total	101(100)	123(100)	93(100)	79(100)	137(100)	92(100)	625(100)	LSD=15.07
St.D	26.6	27.4	18.4	13.9	27.8	10.3		
X^2: 35.61; p = 0.002								

Key: U1= Orlu urban, U2= Owerri urban, U3= Okigwe urban, R1= Orlu rural, R2= Owerri rural, R3= Okigwe rural, LSD=least significant difference.

Malaria Parasitemia among the Respondents

Table 2 depicts malaria parasitemia among the respondents, it was shown that in malaria parasitemia of the respondents, majority of the respondents 395(64%) were malaria positive compared to least 288(36%) who were negative. Higher malaria parasitemia was recorded in rural area 203(32.5%) compared to urban area 194(31%). Moreover, owerri zone 173(28%) had more malaria subjects than other senatorial zones with significant association (p = 0.018). In addition, the result of the parasitemia suffered by the respondents as shown in Table 2 revealed that almost 358(90%) all of them suffered *plasmodium faciparium* compared to least 39(10%) that suffered *plasmodium vivax*. Invasion of this specie was more 159(%) in Owerri zone compared to other zones. Considering the malaria parasitemia, majority of the subjects 246(62%) had low malaria parasitemia compared to those that had moderate 95(24%) and high 54(14%) malaria parasitemia. Significantly (p<0.05) high 28(7%), moderate 42(11%) and low 103(26%) level of malaria parasitemia was recorded in Owerri zone compared to other zones.

Table 2: Malaria Parasiteamia among the respondents

	U1	U2	U3	R1	R2	R3	TOTAL	ANOVA
Malaria infection								
Positive	49(49)	86(70)	59(63)	52(66)	87(64)	64(70)	397(64)	66
Negative	52(51)	37(30)	34(37)	27(34)	50(36)	28(30)	228(36)	38
TOTAL	101(100)	123(100)	93(100)	79(100)	137(100)	92(100)	625(100)	LSD=24.27
St.D	2	35	18	18	26	25		
X^2: 13.62; p = 0.018								
Parasitaemia								
Plasmodium faciparium	44(90)	80(93)	57(97)	39(75)	79(91)	59(92)	358(90)	60
Plasmodium vivax	5(10)	6(7)	2(3)	13(25)	8(9)	5(8)	39(10)	7
Total	49(100)	86(100)	59(100)	52(100)	87(100)	64(100)	397(100)	LSD= 23.43
St.D	27.6	52.3	38.9	18.4	50.2	38.2		
X^2: 17.4; p = 0.004								
Malaria Parasiteamia								
Low	29(59)	51(59)	39(66)	37(71)	52(60)	38(59)	246(62)	41
Moderate	12(24)	25(29)	14(24)	13(25)	17(20)	14(22)	95(24)	16
High	8(16)	10(12)	6(10)	2(4)	18(21)	12(19)	56(14)	9
TOTAL	49(100)	86(100)	59(100)	52(100)	87(100)	64(100)	397(100)	LSD= 7.608
St.D	11.2	20.7	17.2	17.9	19.9	14.5		
X^2: 11.69; p = 0.306								

Key: U1= Orlu urban, U2= Owerri urban, U3= Okigwe urban, R1= Orlu rural, R2= Owerri rural, R3= Okigwe rural, LSD=least significant difference.

Association between respondents' ABO blood group and Malaria parasitemia

Table 3 depicts the association between respondents' blood group and malaria parasitemia. Significant association (p<0.001) existed between blood group and malaria parasitemia. As shown in the results, out of the 397 respondents that had malaria, majority of them who belonged to O blood group had more low malaria parasitemia 118(30%) compared to A group 88(22%), B 32(8%) and AB 8(2%) blood group. Similarly, more of the respondents who belonged to O blood group had more of moderate malaria parasitemia 41(10%) than A 33(8%), B 15(4%) and AB 6(2%) blood group. Also, subjects with O blood group 28(7%) had high of malaria parasitemia compared to A 26(7%) and B 2(1%) blood group. In general, respondents with O group 187(47) had malaria parasitemia compared to A 147(37), B 49(12) and AB 14(4) group.

Table 3: Association between respondents' blood group and malaria parasitemia

Blood group	Malaria severity	Frequency
0	Low	118(30)
	Moderate	41(10)
	High	28(7)
TOTAL		187(47)
St.D		49
A	Low	88(22)
	Moderate	33(8)
	High	26(7)
TOTAL		147(37)
St.D		34
B	Low	32(8)
	Moderate	15(4)
	High	2(1)
TOTAL		49(12)
St.D		15
AB	Low	8(2)
	Moderate	6(2)
	High	0(0)
TOTAL		14(4)
St.D		4.2
X^2: 426.71; p < 0.001		

Discussion

According to [31] socio-demographic attributes play a major role in the health of an individual and the community and hence need adequate consideration in public health intervention strategies. The respondents were dominated by male 331(53%) youths who were within the age group of 31-40 years 152(24%). In the study of [32] in Jhansi, India, almost 75% of all patients were in younger to middle age group (i.e. between 18-40yrs of age). Younger people within the age bracket of 31-40 years were mostly residing in urban area 100(16%) while older people within the age bracket of 51-60 mostly reside in the rural area 72(12%). Most of them had primary school education 219(35%) as their highest level of education. The effects of education can be considered from different perspectives. One such perspective being the height of knowledge that the household may have in terms of malaria transmission patterns, and the other perspective is the extent of literacy of the head of the household [33] and members of the household, and its impact on the knowledge of malaria intervention information and the strategies thereof. Almost half of them 296(47%) belong to the O blood group. According to [34], The ABO blood system is assumed to play a critical role in the protection against intense malaria. The ABO blood group antigen has 3 alleles namely A, B and O. It codes for varying kinds of agglutinogens attached to the surface of red blood cells (RBCs) and thus determines a person's blood group. Blood group frequency in this study (O>A>B>AB) was dissimilar to the ABO phenotypic distribution recorded by [35] and [36] in the region of Balochistan and Pakistan respectively where the most common being O, followed by B, A, and AB. In terms of the malaria parasitemia, majority of the subjects 246(62%) had low malaria parasitemia compared to those that had moderate 95(24%) and high 54(14%) malaria parasitemia. Significantly ($p<0.05$) high 28(7%), moderate 42(11%) and low 103(26%) level of malaria infection was recorded in Owerri zone compared to other zones. This results contradicts the findings of [37] in North West Region of Cameroon where they reported that among the malaria positive cases, 74.38% (119/160) had mild parasitamia, while, only 10% (16/160) had moderate parasitamia, 15.62% (25/160) had severe parasitamia. All human beings are equally susceptible to anopheles mosquito bite. Some people develop mild type of disease whereas others suffer severe symptoms [38]. In recent times, the potential effects of demographic variables and environmental factors malaria renascence and local transmission are however, becoming more vital topics of discussion [39].

Blood group had significant influence ($p=0.022$) on malaria infection such that participants belonging to O blood group 187(47%) had more malaria than other blood group. Previously, [40] reported that *Anopheles gambie* recognizes ABO blood group differences with a preference for blood group O. It is obvious that this observation might have contributed to the high percentage of blood group O infected with malaria. The abundance of O blood group in the study may also be a factor for the high prevalence [41]. This relationship is in line with the observed preponderance of blood group O in malaria endemic sub-Saharan Africa compared to other parts of the world where malaria is not endemic [27].

Association between blood group and malaria parasitemia was significant, it showed that low malaria parasitemia was mostly experienced by the malaria subjects irrespective of blood group, although malaria was prominent in O blood group 187(47%), it was mainly at low severity rate whereas, equal high malaria severity (7%) was found in subjects with blood group of O and A. This was in agreement with several researches that observed low parasitemia and uncomplicated malaria incidence among individuals with blood group O [42, 43]. In the reports of [44, 45], blood group AB individuals had the lowest malaria attack whereas blood group A individuals had the highest malaria infection. Studies have demonstrated that the blood group A has been identified as a risk factor in acute malaria [49] in non-pregnant women while in this group of people, the blood group O persons have been linked with reduced prevalence of acute clinical malaria [46, 47].

The blood group A has been linked with an increased risk factor in severe malaria, meanwhile, blood group O may provide some protection against disease severity [48]. In contrast, various ABO blood systems in the study of [49] showed no serious difference when compared to one another. For more details on malaria, see [50, 51, 52, 53, 54].

Conclusions

Malaria parasitemia in the study area was high particularly in the rural area. Owerri zone had significantly higher (28%) malaria subjects than other senatorial zones. Majority of them had low malaria parasitemia and almost all of them suffered *plasmodium faciparium*. Malaria parasitemia was significantly higher in subjects belonging to O blood group.

Study Limitations

This research did not aim at explaining the mechanism of protection that ABO blood group arguably confers against malaria attack.

The present study only used parasitemia as a laboratory marker to demonstrate the influence of ABO blood groups on malaria parasitemia. The study also did not consider other factor like, place of residence of the study population which could affect intensity of malaria parasitemia. More valuable information would have been generated on the associations had it been more laboratory markers or clinical features (e.g. cerebral malaria) were employed.

Author's Contribution

UMU: Data collection, performed the statistical analysis, interpreted the data and drafted the manuscript. BEBN: Supervised the study, Study design, and revisited the manuscript and critically evaluated the intellectual contents, SJO: Supervised the study, Study design and revisited the manuscript and critically evaluated the intellectual contents. All authors read and signed the final version of paper

Ethical Considerations

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors

Competing Interests

The authors declare that they have no competing interests.

Consent for Publication

Not applicable.

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