

# THE PREVALENCE OF MALARIA IN COVID-19 PATIENTS AMONG BLOOD GROUP CLASSIFICATION

## Abstract

### Background

COVID-19 is caused by SARS-CoV-2; it is considered a global tragedy that has astounded health care systems. Since the pandemic began, identifying the characteristics that might influence risk of infection and poor disease outcomes have been of paramount interest. Malaria is still a major global health burden particularly in the sub-Saharan Africa region with about 230 million cases annually. Blood group systems are genetically inherited features whose association with certain infectious diseases have long been debated.

### Aim

This observational study was aimed at evaluating the prevalence of malaria in covid-19 patients based on blood group in Rivers State Isolation Covid-19 centers located in Rivers state Nigeria.

### Methodology

400 subjects participated and gave consent to be included in the study. Subjects were grouped ABO blood classification using commercially available anti-sera A, B. Microscopic method was used for the examination of malaria parasite and Giemsa staining technique.

### Results

Out of the 400 samples, 327 subjects were infected with malaria parasite while 73 were uninfected. Furthermore, 91 subjects were blood group A, 85 subjects were blood group B, 10 subjects were blood group AB and 214 subjects were blood group O. Additionally, the number of subjects infected with malaria among blood group A, AB, B and O are 71, 69, 7 and 180 respectively. Also the distribution of covid-19 among blood group A, B, AB and O is 78.02%, 81.17%, 70.00%, and 84.11% respectively. Furthermore, the prevalence of malaria and Covid-19 infection based on blood A, B, AB and O appeared to be 14.28%, 9.41%, 0.00%, 16.82% respectively.

### Conclusion

This study has shown that most COVID-19 patients in Rivers state COVID-19 centers have blood groups O and A and had malaria co-infection. The prevalence of malaria with COVID-19 patients based on blood group was high amongst group O patient.

*Key Words: Prevalence, Covid-19, Malaria, Blood Group*

## 1.0 Introduction

The Corona Virus Disease of 2019 (COVID-19) is a new coronavirus that causes acute respiratory disease. Its discovery was in December 2019 in Wuhan, China. There was a rapid increase in the Cases of COVID-19 within few weeks leading to global epidemic by March 11, 2020 [1]. The cause of the disease is severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which has led to a global pandemic to date, Considerable rate of morbidity and mortality has been caused by CoV-2 globally, it has brought about more than 236 million cases and 4.83 million deaths which was reported as at Oct 8, 2021. The first reported cases of SARS-CoV-2 in Africa was on Feb 14, 2020 and has led to unprecedented socio economic and health-care system disruptions in all countries in the continent [2]. The occurrence of COVID-19 pandemic stimulated exceptionally modern crisis [3]. The course of the disease is different among individuals, it can be from mild or even subclinical infection to very severe disease. Scientists are

still curious of the potential risk factors that may affect predisposition to infection and disease progression is still. But there are established medical and sociodemographic risk factors for severe outcomes [3].

Unicellular protozoan from the *Plasmodium* genus causes malaria infection. The following species of *Plasmodium* are known to infect humans; *ovale*, *vivax*, *malariae*, *falciparum*, and *knowlesi* [4]. Malaria remains a major global health challenge, despite the many strategies that has been put in place to eradicate malaria it has still remained a global burden, especially in the sub-Saharan Africa region that still records about 230 million cases annually. It has been estimated by World Health Organization (WHO) that 40% of the world's population is at risk of malaria infection, this has made it a major global infectious disease [5].

ABO is the most important system for blood group compatibility in clinical practice. ABO blood group are system of carbohydrate antigens expressed on human red blood cells) and other human cells. "A" and "B" antigens on red blood cells are trisaccharides, all erythrocytes possess "H" disaccharide on their surfaces (except the rare Bombay phenotype, which has no ABO antigens). Individuals with blood groups "A" and "B" have the "A" and "B" antigens, respectively, together with the "H" antigen. Blood group "AB" individuals have both "A" and "B" antigens together with the "H." Blood group "O" individuals, conversely, have neither "A" nor "B" antigens but "H." there exist an association between ABO blood type and malaria and some others like, cholera, tuberculosis, retrovirus, *Helicobacter pylori* (*H. pylori*) *Escherichia coli*, and *Chikungunya virus* [6]. There are numerous principles ranging from simple (e.g. interactions between receptor and ligand) to complex which may be limited to a specific pathogenic product, strain or disease state. A possible explanation for ABO, possible include natural antibodies and lectins as inhibitors, ABH antigens as receptors for pathogens, and molecular mimicry by blood group antigens between pathogen and host. Evolving evidence show that the immunopathogenesis of SARS-CoV-2 infection can be associated with the role played by ABO blood group. Blood group A can confer risks of higher disease susceptibility and severity while blood group O is protective [7].

The severity of Malaria infection has shown to be associated with the ABO blood group system. Individuals having the O blood group are having less susceptibility to getting infected as compared to individuals having other blood group. The mechanism of protection is thought to be the rosette phenomenon, that is generated initially by the parasite proteins thrombospondin-related adhesive protein (MTRAP) and RH5. Rosette-like RBC clusters are formed when MTRAP and RH5 bind to the CD147 receptor on the surface of infected red blood cells (RBCs), making them stickier thereby facilitating their attachment to healthy erythrocytes [8]. The parasite escape recognition by phagocytosis via the help of the Rosettes. In the case of individuals with blood group O the formation of Clump is weakly seen.

A study done by Ikehin 2021 [9] evaluated the association of *falciparum* malaria and ABO Blood group in Awka, Anambra state, Nigeria. Blood group O and A had higher malaria prevalence of

48.38% and 19.35% respectively while blood groups B and AB had a lower prevalence of 0% respectively. The findings of their study showed that individuals with blood group O were susceptible to contracting uncomplicated malaria but had a higher resistance to going down with severe malaria as compared to those of non-O blood groups [9]. Also, a study carried out in Sudan by Taha and his colleagues in 2020 reported that blood group O was the least affected by the disease while blood group A individuals were the most vulnerable [10].

Several studies have been separately done between COVID-19 and ABO blood group, ABO blood group and malaria but very few studies have been done on malaria and ABO blood group system with covid-19 co-infection hence this study. The essence of this research is to study the prevalence of malaria in covid-19 patients based on blood group system.

## **2.0 MATERIALS AND METHOD**

### **2.1 Study Area**

This research is an observational study conducted at Rivers State Covid-19 Isolation Centre Rivers state, Nigeria. Rivers state is located in the south-south geopolitical zone of Nigeria with significant population strength and economic activities.

### **2.2 Study Population**

The study was conducted among 400 Covid-19 subjects (250 males and 150 females). Consenting health facility confirmed patients were COVID-19 positive via their most recent laboratory test result (usually less than 72 hours) in their clinical folder.

### **2.3 Eligibility criteria**

#### **Inclusion Criteria**

The following subjects were included in the study; subjects admitted into the covid-19 centers, covid-19 patients of less than 50 years and greater than (or equal to) 50 years who were admitted in the centre, and subjects that gave informed consent after counseling were all included for the study

#### **Exclusion Criteria**

This study focused on only ABO blood group system, those who were not admitted into the covid-19 center, subjects who recently recovered were all excluded. Only government accredited covid-19 center was used.

### **2.4 Sampling Method**

Simple random sampling method was used in this observational study among subjects admitted to the government covid-19 center. Subjects who met inclusion criteria and gave consent were selected, male and female subjects were selected until the required sample size was achieved.

### **2.5 Specimen Collection**

Blood sample was collected by venipuncture technique [11]. Blood sample were obtained collected by a trained phlebotomist. 5 mL blood was obtained from each participant. After collection, blood samples were transferred into an ethylene diamine tetra acetic acid (EDTA) tube to prevent blood coagulation. Sample was used for malaria parasite estimation and blood group estimation.

## 2.6 Sample Analysis:

### Malaria parasite Estimation

Thick and thin smears were made on well cleaned and sterilized slides, stained and examined

**Thick films:** Large drop of blood was placed at the center of a clean grease-free slide. The smear was made with the edge of another slide to cover an area on the slide. The slide was labeled with a diamond pencil and kept to air-dry. The smear labeled with a grease pencil and kept to dry in the air. This method yields a much higher concentration of the parasites when they are few in numbers [12].

**Thin film:** - A drop of blood was gently touched onto one end of a clean grease-free slide. A spreader was placed at a suitable angle in front of a blood, and the blood allowed touching and spreading along the edge of the spreader. The spreader was pushed along the slide, drawing the blood behind it, until the whole drop had been smeared. This was labeled and kept to air dry. This method permits the study of the morphology and density of the parasites and the condition of the blood corpuscles [12].

### Staining Technique:

**Giemsa staining method** – thin films were first fixed in absolute methyl alcohol for 2 minutes. The diluted 1:10 Giemsa stain was used to flood both the thin and thick films and left for 45 minutes. The slides were washed in buffered distilled water pH 7.2 and allowed to dry[12].

### Examination

The slides were later viewed under x100 objective lens of the light microscope to confirm the presence or absence of *Plasmodium* parasites and the species present. When about 200 microscopic fields have been observed and no parasite discovered, it is considered negative.

The mean parasite density was classified according to the recommendations of Atroosh in.2015 [13]. Parasite density was recorded as the number of Parasite/ $\mu$ L of blood, assuming an average leucocyte count of 8,000/ $\mu$ L of blood for an average individual [14]. The formula used is stated as follows:

Parasite/ $\mu$ Lblood=Numberofparasitescounted $\times$ 8000whitecells/Ml/  
No.ofwhitecellscounted

### Blood Group Determination

Each subject ABO blood group was typed by agglutination using commercially available anti-sera A, B.

Procedure:

Two drops of whole blood were placed in three different places on a grease-free clean blood group tile

Few drops of blood group A, B and Rhesus factor (D) anti-sera was applied onto each of the three different spots on the blood group tile.

The blood cells and the antigens were mixed with applicator stick.

The tile was then tilted to detect any agglutination

The results were recorded accordingly [15].

### 2.7 Statistical Analysis

Data collected and entered in Microsoft Excel spread sheet. The data were analyzed for descriptive statistics. Prevalence was calculated as the product of the quotient of infected subject and uninfected subject, and 100. The prevalence was expressed in percentage.

### 3.0 RESULT

A total of four hundred (400) blood samples were examined for this study. All study subjects were covid-19 patients, among which were both male and female. Out of the 400 samples, 327 subjects were infected with malaria parasite while 73 were uninfected.

**Table 1: Frequency Distribution of Socio-demographics**

Variable	Frequency	Percentage
Sex		
Female	150	37.50%
Male	250	62.50%
Age		
<50 Years	93	23.25%
≥50 Years	307	76.75%

Table 1 shows frequency and percentage distribution of socio-demographics of the subjects, 150 (37.50%) females and 250 males (62.50%). <50 Years subjects were 93 (23.25%), ≥50 years' subjects were 307 (76.75%).

**Table 2: Frequency Distribution of ABO Blood Group among Study Subjects.**

Blood Groups	Number Examined	Number Infected	Number Uninfected
--------------	-----------------	-----------------	-------------------

A	91	71	20
B	85	69	16
AB	10	07	03
O	214	180	34
<b>TOTAL</b>	<b>400</b>	<b>327</b>	<b>73</b>

Table 2 show the frequency distribution of ABO blood group among subjects.91 subjects were blood group A,85 subjects were blood group B,10 subjects were blood group AB and 214 subjects were blood group O. Additionally,71 out of 91 blood group A subjects were infected with malaria parasite,20 were uninfected. 69 out of 85 blood group B were infected ,16 subjects were uninfected. 7 AB blood group subjects had malaria infection and 3 were uninfected.180 blood group subjects were infected with malaria parasite,34 were uninfected.

**Comment [mrs1]:** Mention blood group → among O blood group 180 subjects were infected with malaria parasite,34 were uninfected

**Table 3: Percentage Distribution of ABO among Covid-19 Patients**

**Comment [mrs2]:** Table2 & table 3 have same title as all study subjects are covid-19 patients.

Table 2 & table 3 are giving same information that number/percentage of malaria infection among different blood groups. Instead it can be explained in one table to show number and percentage.

Table 2&3 title given as-**Frequency/percentage Distribution of ABO Blood Group among Study Subjects**. but data given is number/percentage of malaria parasitemia among ABO blood groups.

Blood Group	Number Examined	Number Infected	Percentage (%)
A	91	71	78.02
B	85	69	81.17
AB	10	07	70.00
O	214	180	84.11
<b>TOTAL</b>	<b>400</b>	<b>327</b>	<b>81.75</b>

Table 3 describes the percentage distribution of ABO blood group among covid-19 patients. Distribution of covid-19 among blood group A, B, AB and O is 78.02%, 81.17%, 70.00%, and 84.11% respectively.

**Table 4: Distribution of Malaria Parasite among Covid-19 Patient**

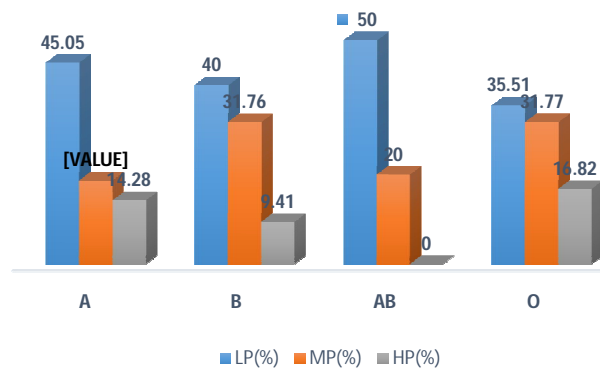
Blood Group	NumberExamined	Malaria Parasitaemia			
		Low	Moderate	High	Total
A	91	41	17	13	71
B	85	34	27	08	69
AB	10	05	02	0	7
O	214	76	68	36	180
<b>Total</b>	<b>400</b>	<b>156</b>	<b>114</b>	<b>57</b>	<b>327</b>

Table 4 shows distribution of malaria parasite among covid-19 patient based on ABO blood group system. The total density of malaria parasitaemia for blood group A, B, AB and O is 71, 69, 7 and 180 respectively.

**Table 5: Prevalence of Malaria Parasitaemiaamong Covid-19 Patient Based on ABO Blood Group**

Blood group	Malaria Parasitaemia		
	Low(%)	Moderate(%)	High(%)
A	45.05	18.68	14.28
B	40.00	31.76	9.41
AB	50.00	20.00	0.00
O	35.51	31.77	16.82

Table 5 defines malaria prevalence among covid-19 patient based on blood group. malaria prevalence and covid -19 infection based on blood A, B, AB and O appeared to be 14.28%, 9.41%, 0.00%,16.82% respectively.



**Figure1: Bar chart shows the prevalence of malaria with covid-19 based on blood group.**

#### 4.0 DISCUSSION

Massive majority of COVID-19 confirmed patients in this study were  $\leq 50$  years old; this is similar with the study done by Remahand his team in 2021 [16]. Also, males appeared more than females, therefore, may be at greater risk of COVID-19 and malaria coinfection than their female counterpart.

In 2021, a systematic review by Wilairatana on prevalence of malaria among covid-19 subjects reported that the prevalence of malaria among covid-19 subjects was 11% among studies in Nigeria, India and Democratic Republic of Congo [17]. Contrarily, this study reported high prevalence of malaria among covid-19 subjects. The prevalence was reported to be 81.75%. There are similarities in the clinical presentation of both malaria and COVID-19 that has made

it difficult to differentiated the two diseases, especially in areas were malaria is endemic [18]. This has made the differential diagnosis of malaria and COVID-19 very challenging.

Covid-19 and malaria prevalence in relation to blood group was highest among blood group O subjects than all other blood groups in this study which is similar to the research carried out by Ramah and his colleagues but contradicts the research carried out by Padhi [19] in India. The findings of Padhi and colleagues had blood group A as the most susceptible to COVID-19 while blood group O appeared less frequently.

It has been postulated that some factors play significant role in the low prevalence of COVID-19 in malaria endemic countries, these include; Angiotensin-converting enzyme 2 (ACE2), hydroxychloroquine (HCQ) and chloroquine (CQ), interferons and the neutralizing antibodies [20]. The interaction between the spike in protein of SARS-CoV-2 and ACE-2 receptor is altered by the ABO but the mechanism is still unknown. There is a release to infect other host as SARS-CoV-2 multiplies in the host cell, depending on the blood group of carriers the spike protein of virions possibly carries A, B, or AB glycan antigen. The infection rate of SARS-CoV-2 and malaria load with A, B, or AB antigens is usually reduced. Since subject with blood group O carries both antibodies A and B [21].

According to Mazda, ABO antibodies titer, which correlate inversely with the degree of industrialization may play a role in the degree of protection against SARS-CoV-2 with malaria infection [22]. Possibly, COVID-19 mortality is reduced significantly by higher occurrence of blood group O type in malaria-endemic regions. The low mortality rate in those geographical areas could be as a result of higher prevalence of blood group O in malaria-endemic areas. The highest prevalence in this study area was Blood group O as it appeared more frequently.

Previous studies have established a strong positive correlation between malarial parasitic density and severity of malarial infection [23]. This study has shown that low parasitic density of malaria was the most prevalent in covid-19 subjects across the blood groups. Every blood group presented with highest low density malarial parasitaemia, followed by moderate density of malarial parasitaemia and the least of all the prevalence among the covid-19 subjects was high density malarial parasitaemia. This pattern is consistent with the studied ABO blood groups.

## **CONCLUSION**

This study has shown that the prevalence of malaria among Covid-19 patients is high in Rivers State and as such the tendency of malaria-covid-19 co-infection is high among covid-19 patients. This is particular among blood group O subjects. Although comorbidities between malaria and Covid-19 existed but less frequently was co-mortality due low degree of high malarial parasitaemia, the burden of covid-19 singularly causes severe trauma on infected individuals locally and globally, hence an increased burden from malarial co-infection should be discouraged in Rivers State. The report from this study calls for immediate public health

response to fight against the co-infection emergence especially in a malaria endemic country like Nigeria.

### **RECOMMENDATION**

More public health responses are needed to curb the spread of malaria in the state. The authorities are required to ensure that the state Covid-19 isolation centre is free from mosquitoes.

### **CONSENT**

The aim of the research was made clear to the Covid-19 patients through health workers that took care of them.

### **ETHICAL APPROVAL**

Before this study commenced, ethical clearance was obtained from the Rivers State Health and Ethics Committee.

### **REFERENCES**

1. Sohrabi, C., Alsafi, Z., Neill, O., Khan, N., Kerwan, M., Al-Jabir, A., Iosifidis, A., Agha, R. (2020). World Health Organization Declares Global Emergency: A Review of the 2019 Novel Coronavirus (COVID-19). *Int J Surg (London, England)*. 76:71–76.
2. United Nations, (2020). Impact of COVID-19 in Africa. (accessed March 4, 2021) <https://unsdg.un.org/sites/default/files/2020-05/Policy-brief-Impact-of-COVID-19-in-Africa>.
3. Amir, A., Cheong, F. W., de Silva, J. R., Liew, J. W. K., Lau, Y. L. (2018). Plasmodium knowlesi malaria: current research perspectives. *IDR*, 11, 1145.
4. Lucky, A. B., Sakaguchi, M., Kataaai, Y., Kawai, S., Yahata, K., Templeton, T. J., et al. (2016). Plasmodium knowlesi skeleton-binding protein 1 localizes to the 'Sinton and Mulligan' stipplings in the cytoplasm of monkey and human erythrocytes. *PLoS ONE*, 11(10), e0164272.
5. World Health Organization (2016). World malaria report 2015. World Health Organization. <https://apps.who.int/iris/handle/10665/200018>.
6. Cooling L: (2015) Blood groups in infection and host susceptibility. *Clinical Microbiology* 28:801–70.
7. Alghanmi, M., Zawawi, A., Alsaady, I., Gattan, H., Zakai, H., Couper, K., (2020). The impact of COVID-19 pandemic on malaria elimination. *Parasite Epidemiology Control*. <https://doi.org/10.1016/j.parepi.2020.e00187>.

8. Handel, I.G., Rowe, J.A., Thera, M.A., Deans, A.M., Lyke, K.E., Kone, A. (2007) Blood group O protects against severe Plasmodium falciparum malaria through the mechanism of reduced rosetting. *Proc Natl Acad Sci U S A*. 2(104)17471–17476.
9. Ikeh, I., Achufusi, I. S., Aribodor, O., & Okeke, O. (2021). Association of Falciparum Malaria and ABO Blood Group in Awka, Anambra State, Nigeria. *Tropical Journal of Applied Natural Sciences*, 3(2), 9–13. <https://doi.org/10.25240/tjans.v3i2.2>.
10. Taha, A., Osman, E., Abdoelkarim, A., Holie, A., Elbasheir, M., Abuzeid, et al (2020) Individuals with a Rh-positive but not Rh-negative blood group are more vulnerable to SARS-CoV-2 infection: *New Microbes and New Infections*, 38:100763, 2052- 2975, <https://doi.org/10.1016/j.nmni.2020.100763>.
11. World Health Organization. (2010) WHO guidelines on drawing blood: best practices in phlebotomy. *World Health Organization*. <https://apps.who.int/iris/handle/10665/44294>
12. Monica Cheesbrough. (2010) District Laboratory practice in Tropical Countries part 2 edition. Cambridge University Press, New York pp 320- 329.
13. Atroosh, W.M., Al-Mekhlaf, H. M., Al-Jasari, A., Sady, H., Al-Delaimy, A. K., Nasr, et al. (2015) Genetic variation of pfrp2 in Plasmodium falciparum isolates from Yemen and the performance of HRP2-based malaria rapid diagnostic test. *Parasites Vectors*, 8, p. 388.
14. WHO Coronavirus Dashboard (2015). Available from: <https://covid19.who.int/>. Accessed 28 March 2022. World Health Organization. Microscopy for the Detection, Identification and Quantification of Malaria Parasites on Stained Thick and Thin Blood Films in Research Settings, p. 32.
15. Monica Cheesbrough. (2002) District Laboratory practice in Tropical Countries part 2 edition. Cambridge University Press, New York.
16. Remah, A., Hassan, R.A., Abdelrahim, N.A., Modawe, G., Enan, K. A. (2021). ABO/Rhesus Blood Groups and Comorbidities of COVID-19 Confirmed Patients Attending Gabra Isolation Center in Khartoum, Sudan. *JUMJ*, 8(4), 23-31.
17. Wilairatana, P., Masangkay, F. R., Kotepui, K. U., Milanez, G. D. J. and Kotepui, M. (2021). Prevalence and characteristics of malaria among COVID-19 individuals: A systematic review, meta-analysis, and analysis of case reports. *Plos Negl Trop Dis*, 15(10): e0009766.
18. Hussein, M.I.H., Albashir, A.A.D., Elawad, O.A.M.A. et al. (2020) Malaria and COVID-19: unmasking their ties. *Malaria Journal* 19, 457. <https://doi.org/10.1186/s12936-020-03541>.
19. Padhi, S., Suvankar, S., Dash, D., Panda, V. K., Pati, A., Panigrahi, J., & Panda, A. K. (2020). ABO blood group system is associated with COVID-19 mortality: An epidemiological investigation in the Indian population. *Transfusion clinical biology*, 27(4), 253–258. <https://doi.org/10.1016/j.tracli.2020.08.009>.

20. Ondoa P, Kebede Y, Loembe MM, Bhiman JN, Tessema SK, Sow A, et al. (2020) COVID-19 testing in Africa: lessons learnt. *Lancet Microbe*. 1:103–4.
21. Breiman, A., Ruvën-Clouet, N., Le Pendu, J. (2020) Harnessing the natural anti-glycan immune response to limit the transmission of enveloped viruses such as SARS-CoV-2. *PLoS Pathogen* 16: e1008556.
22. Mazda, T., Yabe, R., NaThalang, O., Thammavong, T., Tadokoro, K. (2007) Differences in ABO antibody levels among blood donors: a comparison between past and present Japanese, Laotian, and Thai populations. *Immunohematology*, 23:38–41.
23. Kotepui, M., Piwkham, D., PhunPhuech, B., Phiwklam, N., Chupeerach, C. and Duangmano, S. (2015). Effects of malaria parasite density on blood cell parameters. *PloS ONE* 10(3): e0121057.

UNDER PEER REVIEW