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Research Article

Association of ABO Blood Group/Rhesus Factors in Malaria Susceptible Patients in Keffi, Nasarawa State

Ene E. Okon*,[1], Jamey P. Mairiga*, Charity U. Akwa, Favour K. Adams*, Nneka E. Ezema*, Osekweyi I. Odonye*, Bawa. Y. Muhammad*,

[1]*Department of Biochemistry and Molecular Biology, Faculty of Natural and Applied Sciences, Nasarawa State University, Keffi, Nigeria*
[2] *Department of Environmental Education, Faculty of Education, University of Calabar, Etagbor, Nigeria*

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CORRESPONDENCE
Okon, E. E.
ene1990okon@gmail.com
+234-907-192-6121

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ABSTRACT

Malaria is a major cause of death within the sub-Saharan Africa, including Nigeria. This study was aimed to investigate the association among the ABO/Rhesus blood group with individuals that are susceptible to malaria infection in Keffi Local Government Area (LGA), Nasarawa State, Nigeria. A total of 150 subjects confirmed with different degrees of *Plasmodium falciparum* parasitaemia were used for this study. The blood samples of malaria patients were analysed by thin and thick blood films, and their blood group types determined using the tile method. The results showed a higher prevalence of malaria in females (64.7%) than males (35.3%). The age group 31-40 years had the highest prevalence (25.3%). The ABO/Rhesus blood group types revealed the following pattern of distribution for malaria positive individuals: ORh+ > ARh+ > BRh+ > ABRh+ > ORh- > ARh- with the following distribution (44.7%), (21.3%), (18.7%), (6.7%), (4.7%) and (4.0%) respectively. Although ORh+ was the most frequent blood group among malaria-positive individuals, no statistically significant association was found between blood group and malaria susceptibility. It is however important to state that the high prevalence of blood group O among malaria patients may be reflective of the population's blood group distribution rather than increased susceptibility to malaria.

Keywords: *Malaria Parasite, Rhesus Blood group, Susceptibility, Prevalence, Parasitaemia*

INTRODUCTION

Malaria remains a menace and major cause of morbidity and mortality worldwide with an estimated 627,000 deaths in 2021 (WHO, 2022), though this represents a small fraction of the over 200 million clinical cases that occur every year. Gavi, the Vaccine Alliance, stated that new data from the World Health Organisation (WHO) revealed that an estimated 263 million malaria cases and 597,000 malarias deaths occurred worldwide in 2023 (WHO, 2024). This marks an increase of approximately 11 million cases when compared to 2022, with nearly the same number of deaths reported (WHO, 2023). Alarmingly, about 95 percent of these deaths were concentrated in the WHO African region, where many individuals at risk still lack access to essential services for prevention, detection, and treatment of malaria (WHO, 2024). Malaria is transmitted throughout Nigeria, with 97% of the population being at risk of being infected with malaria. According to the 2022 World Malaria Report, Nigeria accounts for the highest percentage of the global malaria burden

compared to any other country, with 27% of the global estimated malaria cases and 31% of the estimated deaths, as well as an estimated 55% of malaria cases in West Africa in 2022 (WHO, 2023). Nigeria's large population, along with other factors such as sanitation, management and vegetation that favours mosquito breeding, account for the persistent rise in malaria transmission (WHO, 2023).

The duration of the transmission season ranges from year-round transmission in the south to three months or less in the north. *Plasmodium falciparum* is the predominant malaria species. The primary vectors across most of the country are An. coluzzii (59.3 percent) and Anopheles (An.) gambiae s.s. (39.0 percent) of all the An. gambiae s.l. collected, with An. funestus as a secondary vector in some areas of Nigeria (WHO, 2023). Microscopy data from the 2018 Nigeria Demographic and Health Survey (NDHS) showed that the prevalence of malaria parasitaemia in children under five years of age is 23% (a decrease from 27% in 2015 and 42% in 2010), although there are significant regional, rural-urban, and socioeconomic differences: prevalence ranges from 16% in the South and South East Zones to 34% in the North West Zone (WHO, 2023). In rural populations, prevalence is 2.4 times than in urban populations (31% vs. 13%). Compared to the highest socioeconomic group, prevalence among children in the lowest socioeconomic group is seven times higher (38% vs. 6%) (WHO, 2023).

According to Dawaki et al., (2016), 3.2 billion people worldwide are at risk of contracting malaria, with Nigeria responsible for 11.0% of maternal deaths and 30% of child deaths, particularly among children under the age of five. Children under the age of five are particularly vulnerable to contracting malaria since they have not yet built-up immunity to the illness (Abossie et al., 2020). Children who survive malaria may experience long-term effects from the infection. Frequent episodes of fever and illness impair growth by decreasing appetite, limiting playtime, social interactions, and educational opportunities. Malaria is known to cause a significant part of child deaths, which are more common in households with lower incomes (Abossie et al., 2020).

Among the species of Plasmodium that infect humans, P. falciparum is the most notorious, followed by P. vivax, with weaker contribution by *P. ovale, P. malariae, and P. knowlesi* (Okagu et al., 2022). Upon injection into the host, MPs (sporozoites) flow through the bloodstream and migrate into the human host's liver and infect the hepatocytes. They then develop into schizonts and release many merozoites into the bloodstream to infect erythrocytes. Most of the pathological effects of malaria infection are accrued to the erythrocytic life cycle stage (Chandley et al., 2023).

The prevalence of malaria amongst children 6 – 59 months was 27% with a slight decline to 23% in 2018 (NMIS, 2015; NPC, 2019). Many studies have shown that the malaria burden is generally higher in rural compared to urban areas (Amodu and Uchendu, 2014). The prevalence of malaria is higher in the Northern part of the country.

According to NDHS 2018, Nigeria's prevalence rate was 22.6% while Nasarawa state reported a 13.6% prevalence rate (Herriman, 2023). Nasarawa state is one of the states in the north-central that is supported with different interventions by various developmental partners/non-governmental organizations in malaria.

Despite the fact that previous studies have shown that malaria is highly prevalent throughout Nigeria (Ibekwe et al., 2009; Gajida et al., 2010; Nmadu et al., 2015; Onyiri, 2015), there is still a dearth of information on the prevalence of the disease in some regions of the nation, particularly among individuals in Nasarawa State, Nigeria.

Many researches have implicated the blood group type to malaria's susceptibility in patients especially in endemic populations. The ABO blood group is the most important among human blood group systems and consists of complex carbohydrate moieties at the extracellular surface of red blood cell (RBC) membrane (Storry and Olsson, 2009; Yamamoto et al., 2010). The ABO blood grouping system is divided into four types namely; A, B, AB, and O (Reid et al., 2012). While the A and B alleles of the ABO locus encode A and B glycosyltransferase activities, which convert precursor H antigen into either A or B determinants by adding an extra saccharide unit, blood group O individuals lack such transferase enzymes and express basic, unchanged H-antigen (Lowe, 2011).

The Rhesus blood group system is the next most clinically significant after the ABO blood group system. There are 5 antigens of the Rhesus blood group system (C, D, E, c and e). The most immunogenic of the antigens of Rhesus D blood group system is the D antigen. These antigens are inherited from biological parents. If the D antigen is present on the red cell surface, the Rhesus D blood type is considered Rh- D positive. Conversely, if the protein is absent, the blood type is Rh- D negative. It is important to state that most people, approximately 85%, have Rh- D positive (Urbaniak and Greiss, 2000).

Blood groups distribution have been extensively studied in a variety of populations around the world, and their frequencies showed significant regional variation both in age and gender, reflecting the underlying genetic and ethnic diversity of human populations (Garratty et al., 2004). Studies conducted elsewhere on patients with malaria have shown that there is an association between malaria and blood groups of an individual (Obisike and Makwe, 2020). These studies have indeed shown that people with the blood group O have a selective evolutionary advantage over malaria compared to people with non- O blood groups (A, B and AB) (Uneke et al., 2006). The mechanism by which blood group O confers selective resistance to malaria, while individuals with non O blood groups show selective vulnerability, has been attributed to rosette formation (Doumbo et al., 2009). Rosetting is a mechanism in which an infected erythrocyte with Plasmodium falciparum adheres to non-infected erythrocytes (Juillerat et al., 2011). Therefore, in patients with malaria with the non-O blood group, invasion of uninfected red blood cells is enhanced, compared to the other red cell polymorphs. Rosetting may be mediated by a membrane protein, including PfEMP1 (Moll et al., 2015), RIFIN and STEVOR (Doumbo et al., 2009), which are expressed on the surface of the infected

host cell. Rosettes formed by non-O blood groups are resilient to disruption (Hedberg et al., 2021).

However, there are several unanswered questions and unclear links about how the various blood groups contribute to severe anaemia presentations. Furthermore, the role of Rhesus (Rh) types in P. falciparum infection remains unanswered, since available data on this subject are equivocal (Yeda et al., 2022).

Despite the comparative vulnerability of blood groups A, B and AB to malaria, the association of these blood groups with malaria severity has not yet been fully studied and understood. Plasmodium parasites are known to cause fever in patients with malaria (Cowman et al., 2016). However, it is interesting to note that the degree of fever, assessed by measuring body temperature, does not always correlate with Plasmodium parasitaemia. Furthermore, patients with malaria have had a different set of clinical signs and symptoms, even with similar parasitaemia (Aninagyei, 2020; Aninagyei et al., 2022). Based on the foregoing, it is hypothesized that host genetic variants may influence clinical malaria presentations. To date, it remains unknown whether blood group variability plays a role in the observed differences in similar levels of parasitaemia. This evidence gap merits further investigation. This study is therefore aimed to investigate the association between ABO/Rh blood group type and malaria susceptibility in Keffi, Nasarawa State, contributing to evidence on the potential link between blood group type and malaria risk.

MATERIALS AND METHODS

Study design

The study was carried out in Keffi local government area in Nasarawa State, Nigeria. Keffi is 50 kilometers from Abuja the Federal Capital of Nigeria and is known to have a population of 92,664 as at 2006 population census (Wikipedia, 2015). In this study, a total of 208 participants whose consent were obtained agreed to participate, out of which only 150 were ascertained to be positive to different levels of parasitaemia of malaria infection and were therefore recruited for the study. They were made up of males and females with their age range between the ages of 1-70 years.

Ethical approval and consent

Ethical approval was obtained from the ethics and grievances committee of the Federal Medical Center Keffi (Ref No: FMC/KF/HREC/004021) while oral consent was obtained from the subjects who offered to participate in the study upon meeting the set criteria for selection.

Sample collection and analysis

Blood samples were collected by trained Medical Laboratory Scientist in the hospital lab using venipuncture technique. The arms of the patients were tied with tourniquet and the position of the veins disinfected using swab soaked in methylated spirit. Blood samples were collected into Ethylene Diamine Tetra Acetic acid (EDTA) tubes and transported to the haematology unit for analysis. Sterile techniques and disaposable, single use materials were used at all times.

Malaria parasite detection and count

Thick and thin blood film smear were made to determine the parasite density as described by WHO criteria for malaria parasite density (WHO, 2000; Cheesebrough, 2005).

Changes in parasitized red blood cells help to identify *plasmodium* species and to detect mixed infection of malaria parasite. The number of asexual P. *falciparum* and other species per 100 leukocytes were counted and if ten or more parasites were identified, then the number was recorded, a blood sample was regarded as negative if the examination of thick films failed to show the presence of asexual parasites. The parasite count in relation to the leukocyte count was converted to parasite per micro liter of blood (assuming 6000 leukocytes per micro liter of blood) using the formula below:

Parasites/µlof blood = No. of parasites counted x 6000.

$$Parasites/\mu l\ of\ blood = \frac{No.\ of\ parasites\ counted \times 600}{No.of\ leukocyte\ counted}$$

Analysis of ABO/Rherus blood group types

The blood group was determined using the tile method as described by Pimenta *et al.,* (2012). Blood group determination ABO blood groups was typed by agglutination using Monoclonal® (Anti serum-A, Anti serum-B and Anti serum-D). Three drops of whole blood were placed in three different places of a grease-free clean tile on which a drop of antisera A, B and Rh were added. The blood cells and the antigen were mixed with applicator stick. The tile was then rotated to detect for agglutination and the result was recorded accordingly.

Statistical analysis

All data were inputted into Microsoft Excel then exported to SPSS version 21.0 (SPSS, Chicago, IL, USA) for descriptive analysis. Pearson's chi-square test was used to determine the association between, blood group type, demographic factors and the malaria parasite. Graphs were represented using descriptive bar charts to display association where necessary. P values < 0.05 were considered statistically significant at a confidence interval of 95%.

RESULTS

Distribution of demographic characteristics of sampled population

A total of 208 malaria-suspected individuals agreed to participate in this study after providing informed consent. Among the study population, 150 were confirmed with different level of parasitaemia. Of the aforementioned number, 141(94.0%) had mild parasitaemia "mp+", 6(4.0%) had moderate parasitaemia "mp++" while 3(2.0%) had high parasitaemia "mp+++" respectively (Figure. 1a). The gender distribution of the malaria-positive cases was 97(64.7%) females and 53(35.3%) males (Fig. 1b). The distribution of the age group among malaria-infected individuals recorded

17(11.3%) individuals for the age group 1-10 years, 35(23.3%) 11-20 years, 25(16.7%) 21-30 years, 38(25.3%) 31-40 years, 27(18.0%) 41-50 years, 5(3.3%) 51-60 years and 3(2.0%) 61-70 years respectively (Figure. 1c).

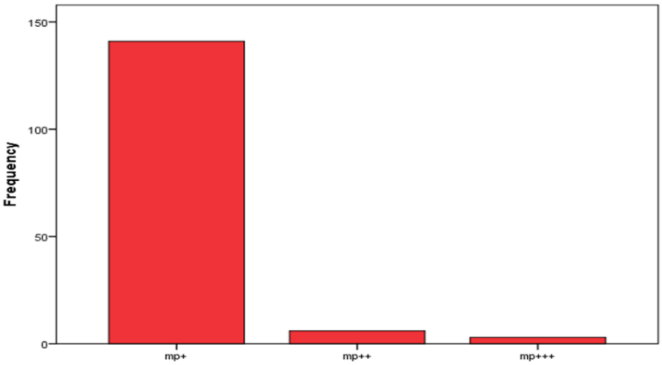

Figure 1a. distribution of malaria parasite based on the level of parasitaemia

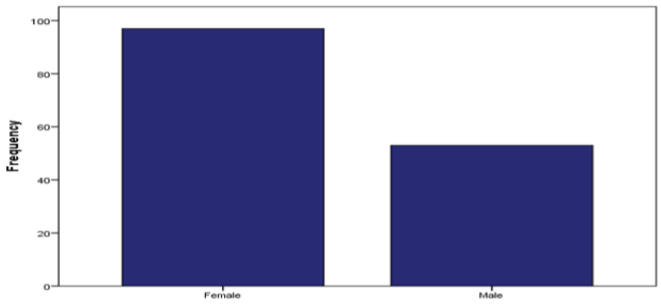

Figure 1b. Frequency of gender of the sample malaria population

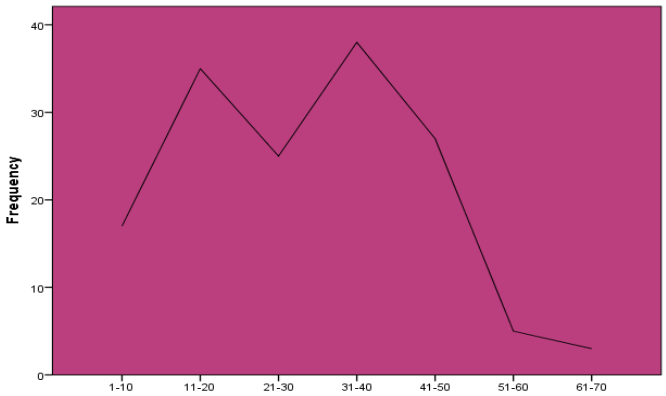

Figure1c. Age range of the sample malaria population

Distribution of the ABO/Rhesus blood group types against susceptibility of individuals to the different level parasitaemia

Figure 1d showed the distribution of 141(94.0%) mild parasitaemia "mp+" patients of ABO/Rhesus blood groups as follows; 6(4.3%) ARh-, 31(22.0%) ARh+, 9(6.4%) ABRh+, 24(17.0%) BRh+, 7(5.0%) ORh- and 64(45.4%) ORh+ respectively. The distribution of ABO/Rhesus blood groups in the 6(4.0%) individuals with moderate parasitaemia "mp++" were 1(16.7%) ABRh+, 3(50.0%) BRh+ and 2(33.3%) ORh+ respectively while the distribution of ABO/Rhesus blood groups in 3(2.0%) individuals with high parasitaemia "mp+++" were 1(33.3%) ARh+, 1(33.3%) BRh+ and 1(33.3%) ORh+ respectively. The chi-square value of 7.439 and a non-significant p-value of 0.683 were obtained, indicating no statistically significant association between ABO/Rhesus blood group types and susceptibility to the different levels of parasitaemia.

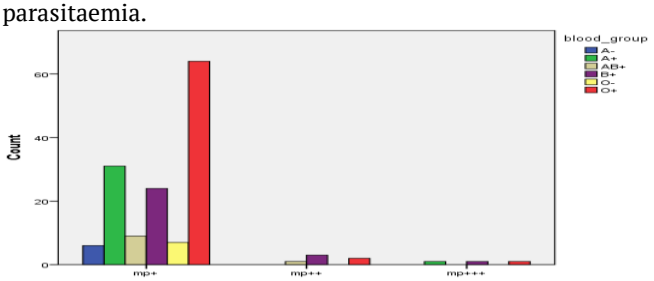

Figure 1d. Distribution of ABO/Rhesus blood group type based on the level of parasitaemia

Distribution of the ABO/Rhesus blood group types among malaria-positive population

The ABO/Rhesus blood group types in figure 1e revealed the following pattern of distribution for malaria positive individuals: ORh+ > ARh+ > BRh+ > ABRh+ > ORh- > ARh- with the following distribution 67(44.7%), 32(21.3%), 28(18.7%), 10(6.7%), 7(4.7%) and 6(4.0%) respectively. The analysis showed that individuals with ORh+ had the highest prevalence of malaria, accounting for 44.7% of the malaria-positive cases in this study.

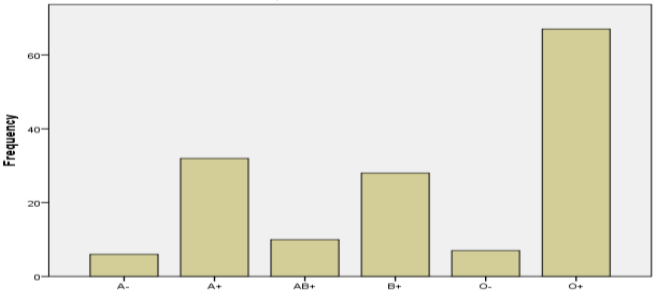

Figure 1e. Distribution of the ABO/Rhesus blood group of the sample population

Gender distribution in the ABO/Rhesus blood group types of the malaria population

There were 97(94.0%) females infected with malaria. Out of this number the distribution of ABO/Rhesus blood groups were 4(4.1%) ARh-, 19(19.1%) ARh+, 7(7.2%) ABRh+, 23(23.7%) BRh+, 2(2.1%) ORh- and 42(43.3%) ORh+, while the distribution of ABO/Rhesus blood groups in 53(35.3%) infected males were 2(3.8%) ARh-, 13(24.5%) ARh+, 3(5.7%) ABRh+, 5(9.4%)BRh+, 5(9.4%) ORh- and 25(47.2%) ORh+ respectively (fig. 1f). A chi-square value of 8.376 and a non-significant p-value of 0.137 were obtained, indicating no statistically significant association between gender and ABO/Rhesus blood group types among malaria-positive individuals.

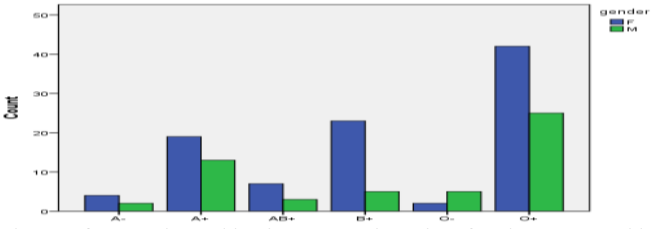

Figure 1f. ABO/Rhesus blood groups and gender of malaria susceptible individuals

Age group distribution of the ABO/Rhesus blood group types of malaria infected individuals

The distribution of ABO/Rhesus blood group types among malaria-infected individuals across different age groups is presented in figure 1g. The outcome indicates that ARh- had 1(16.7%), 3(50.0%), 2(33.3%) for the age group 21-30 years, 31-40 years and 41-50 years respectively. ARh+ had 1(3.1%), 10(31.3%), 8(25.0%), 9(28.1%), 4(12.5%) between the age group 1-10 years, 11-20 years, 21-30 years, 31-40 years and 41-50 years. ABRh+ had 3(30.0%), 4(40.0%), 2(20.0%) and 1(10.0%) between the age group 11-20 years, 21-30 years, 31-40 years and 41-50 years. BRh+ had 5(17.9%), 5(17.9%), 3(10.7%), 5(17.9%), 8(28.6%) and 2(7.1%) between the age group 1-10 years, 11-20 years, 21-30 years, 31-40 years, 41-50 years and 51-60 years. ORh- had 1(14.3%), 2(28.6%), 1(14.3%), 2(28.6%) and 1(28.6%) between the age group 1-10 years, 11-20 years, 31-40 years, 41-50 years and 61-70 years. The blood group ORh+ had the highest outcome with 10(14.9%), 15(22.4%), 9(13.4%), 18(26.9%), 10(14.9%), 3(4.5%) and 2(3.0%) between the age group 1-10 years, 11-20 years, 21-30 years, 31-40 years, 41-50 years, 51-60 years and 61-70 years respectively. A chi-square value of 32.598 and a non-significant p-value of 0.340 were obtained, indicating no statistically significant association between age group and ABO/Rhesus blood group types among malaria patients.

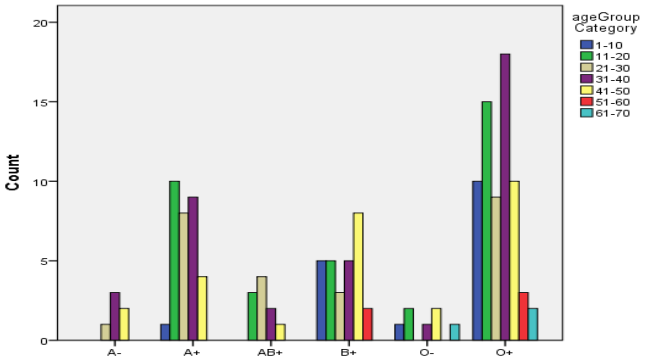

Figure 1g. ABO/Rhesus blood groups and age group of malaria susceptible individuals

DISCUSSION

Variabilities in blood groups play an important role in the pathogenesis of malaria. Several studies have linked malaria susceptibility to non-O blood group (Degarege et al., 2019; Yeda et al., 2022). Among the non-O group, malaria incidence is prevalent among individuals with blood group B (Degarege et al., 2019; Panda et al., 2011). A higher probability of malaria incidence was also reported in blood group AB (Deepa et al., 2011; Zerihun et al., 2011). Olajewo et al., (2013) reported more incidence of malaria infection in blood group O (38.4%), closely followed by A (34.6%), moderate in B (23.1%) and least in AB (3.9%) whereas Gayatri et al., (2013) reported B (40.9%), O (34.16%), A (16.09%) and AB (8.78%). Despite these associations, the impact of ABO and Rhesus (Rh) blood types on malaria has not been fully explored, especially in the Nasarawan population. Here we assess the relationship between the ABO/Rhesus blood groups and the laboratory outcomes of patients with malaria in Keffi local government of Nasarawa State, Nigeria.

In our study, the frequency distribution of ABO/Rhesus blood group types in malaria patients showed O+(44.7%), A+(21.3%), B+(18.7%), AB+(6.7%), O-(4.7%), and A-(4.0%) respectively. This showed that individuals with blood group O+ had the highest frequency of malaria infection, followed by A+, B+, and AB+. According to a recent study conducted by Abriba et al., (2025) in Keffi Local Government, the prevalence of blood groups A, B, AB and O were 28.7%, 22.7%, 3.3% and 45.3% respectively. These values are consistent with the overall prevalence of blood group types in Nigeria, which showed 52.93% for blood group O, 22.77% for blood group A, 20.64% for blood group B, and 3.66% for blood group AB (Anifowoshe et al., 2017). It is possible that the general distribution of blood group types in the study area contributed to the high prevalence of blood group O infected with malaria in our study.

The publication of Nkansah et al., (2023) reported O+(44.39%), B+(27.56%), A+(21.95%) while Aninagyei et al. (2024) recently reported higher frequencies in O+(54.9%), B+(43.3%), A+(37.2%), which is also similar to Onaiwu et al. (2019) who had previously reported higher frequency distribution in O+(58.3%), B+(17.9%), and A+(16.5%). Okon et al., (2025) also supports the high prevalence of O+(46.9%), followed by B+(25.3%) and A+(16.1%) in malaria infection. Notably, A+ was more prevalent than B+ in our study, contrasting with the studies of Nkansah et al., (2023) and Okon et al., (2025) where B+ is greater than A+. Our finding is consistent with previous studies that have reported a higher prevalence of malaria infection among individuals with blood group O (Onaiwu et al., 2019; Nkansah et al., 2023; Aninagyei et al., 2024; Okon et al., 2025). This observation is also consistent with previous publications on this subject matter (Deepa et al., 2011; Panda et al., 2011). Our findings is however contrary to a recent report by Abebe et al., (2024), who reported higher prevalence of malaria infection among individuals with blood group A, followed by those of blood groups B and AB, and the least affected being blood group O+. The observed disparities in prevalence rates may be attributed to variations in vector abundance, malaria seasonality, control measures, study population, and sample size. Epidemiological factors such as variations in vector abundance and distribution can influence the transmission dynamics of malaria, while malaria seasonality can impact the prevalence of infection. Also, differences in control measures, such as insecticide-treated bed net usage and indoor residual spraying, can play a role.

Additionally, biological and environmental factors, including genetic factors (e.g., presence of sickle cell trait) and environmental factors (e.g., climate and proximity to breeding sites), can influence susceptibility to malaria. For instance, the study by Abebe et al., (2024) was conducted in Northeast Ethiopia, which may have unique and peculiar environmental and genetic factors that could possibly influence malaria susceptibility.

The association of blood group B or A with malaria is attributable to rosetting, which is enhanced in these blood groups compared to blood group O (Doumba et al., 2009; Doku et al., 2019). Rosette formation allows uninfected red

blood cells to be attracted to infected red cells, mediated by the parasite protein called Plasmodium falciparum erythrocyte membrane protein (PfEMP1) (Doku et al., 2019). In terms of gender status, more females had malaria infection compared to males which is contrary to recently published report by Abebe et al., (2024), who reported more malaria infection in males than females. The higher prevalence of malaria among females in our study could be attributed to differences in exposure patterns, outdoor activities, or clothing that increase susceptibility to mosquito bites. Also, the hormonal fluctuations in females could potentially explain the gender-based differences in malaria susceptibility. The variations in various studies could be due to genetic factors, prevailing environmental conditions as at the time the research was being carried out and methodology of the study being adopted.

CONCLUSION

Though, our study contributes to the understanding of the relationship between ABO/Rhesus blood group types and malaria susceptibility, however, further research is needed to fully elucidate the mechanisms underlying this association and to explore the potential implications for malaria prevention and treatment.

Limitation of the study

While our study provides key insights into the relationship between ABO/Rhesus blood group types and malaria susceptibility, there are several potential limitations to consider. For instance, our sample size was limited to 150 malaria-positive individuals, which might have impacted our ability to detect significant differences between groups. Additionally, the lack of a control group hinders our ability to compare blood group distributions between malaria patients and healthy individuals.

Furthermore, our study was conducted in just one local government area, Keffi, which might not be a true reflection of the general population. Future studies should aim to replicate our findings in different populations and settings to further explore the relationship between ABO/Rhesus blood group types and malaria susceptibility.

AUTHORS' CONTRIBUTIONS

Conceptualization, EEO. and JPM.; methodology, EEO. and JPM.; validation, CUA, OIO, FKA. and NEE.; formal analysis, EEO. and JPM.; investigation, EEO.; resources, CUA, OIO, FKA. and NEE.; data curation, EEO.; writing—original draft preparation, EEO.; writing—review and editing, JPM, MBY. and CA.; supervision, JPM. and MBY.; project administration, EEO. and JPM.; funding acquisition, CA, OIO, FKA. and NEE. All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST

The authors declare that they have no financial or other relationships that might lead to any conflict

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