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Original Article

ABO Blood Group: Risk Factors For Malaria In Hypo-endemic Areas?

Lambok Siahaan

Parasitology Department Medical Faculty, Sumatera Utara University, Indonesia
E-mail Corresponding: lamboksiahaan_fkusu@yahoo.com

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ABSTRACT

Background: Malaria is a parasitic infectious disease that is transmitted by vectors. This disease can cause death, especially in babies, toddlers and pregnant women. This research was conducted to prove whether there was a relationship between blood group and the occurrence of malaria infection.

Methods: The sample was people who permanently reside in the research area and have risk factors for exposure to malaria. Diagnosis was made through microscopic examination. Blood type was tested using the slide method.

Results: The most common cause of malaria was Plasmodium vivax. There were no significant differences between etiology, clinical symptoms, hemoglobin levels and parasite density with ABO blood group. This study found no significant differences between ABO and malaria blood types.

Conclusion: ABO blood type cannot be used as a risk factor in hypoendemic areas which have the majority of vivax malaria sufferers.

INTRODUCTION

Malaria is still a vector-borne disease that impacts human health and can cause death. In fact, WHO reports that there was a trend of increasing malaria cases after the decline in COVID-19 cases.1 A good intervention strategy must begin by finding risk factors that are very specific for each endemic area. The risk factors for malaria are very dynamic and can change due to many factors.2 Regular identification of risk factors, especially in hypoendemic areas, will help efforts to achieve a malaria-free Indonesia by 2030.

Several studies show that there was a relationship between genetic factors,

including ABO blood type, with human susceptibility to malaria.3 Malaria infection is greatly influenced by the parasite's ability to attach to the walls of red blood cells before entering them. Many factors influenced the ability of parasites to invade red blood cells.

ABO blood group is a blood grouping system that is often used as an important indicator of hemolytic transfusion reactions. Blood grouping was made based on differences in carbohydrate antigens on erythrocytes. The variation in clinical manifestations of malaria sufferers has prompted research to look at the relationship between the patient's clinical condition and their blood type. However, the results found are still relatively contradictory between one study and another. Several studies have shown that the incidence of malaria infection does not vary significantly with the patient's blood type. However, research shows that people with type A blood are more likely to develop malaria. Meanwhile, in other studies, malaria sufferers with blood type O had milder clinical manifestations and even tended to be protected from severe malaria. So it can be concluded that all of this research were carried out to prove whether the ABO blood group antigen had a relationship with the occurrence of malaria, clinical manifestations of malaria and the occurrence of antimalarial resistance.4

Research on malaria and its relationship with blood antigens began several decades ago, but rational reasons are still needed to explain the relationship. This was very necessary to explain and anticipate the occurrence of pathological processes due to the interaction of parasites with red blood cells, especially the attachment of parasites to the surface of erythrocytes before entering them.5 Therefore more such studies are needed to improve understanding of the relationship between blood groups and malaria infection. This study aims to determine the relationship between blood type and the occurrence of malaria in hypoendemic areas. Another aim was to obtain data on the proportion of malaria sufferers based on clinical symptoms, etiology, parasite density and hemoglobin levels.

METHOD

This study was a component of one that looked at developing a diagnostic model for asymptomatic malaria patients in hypoendemic regions. The information presented in this paper was gathered in 25 villages and 4 community health centers in the Batubara Regency of the North Sumatra Province between March 2015 and December 2017. Each research subject was observed for two weeks as part of a longitudinal study (based on the average incubation period for malaria). Each study subject was observed for two periods of malaria during the study interval.

The sample in this study were people who permanently reside around the research area who were selected using simple random sampling. After providing an explanation and obtaining informed consent, the research will continue with examination of the research samples. A microscopic examination conducted by a minimum of two skilled microscopists allowed for the diagnosis of malaria. Blood tests were carried out for three consecutive days on each patient. Submicroscopic malaria infection was established in patients who were negative on the first day and positive on the second or third day. Meanwhile, asymptomatic malaria infection was confirmed in patients who did not have complaints of fever but were positive on microscopic examination on the first day of examination. Measurement of hemoglobin values was carried out using the dipstick method. (Lot HB15414B4T, Easy Touch GHb Meter: Control: (N) 12–15 g/ml). Blood group examination was carried out using the

antigen and antibody agglutination method on slides (CellLine/Clone: 11H5(A), BRIC250(B), BRIC186(AB)). Meanwhile, other characteristics were obtained through interviews and observation.

Both absolute values and percentages (%) were used to represent categorical variables. Standard deviation (SD) and mean were displayed for numerical variables in the interim. The chi-square test was used to assess group differences for categorical variables. Numerical analysis was also subjected to the Analysis of Variance (ANOVA) test. Statistical analysis was performed using statistical software.

RESULT

This research was conducted on 3709 participants as research samples. Research samples were obtained in two ways, namely actively and passively. Active sampling was carried out by visiting an area where based on reports there were cases of malaria in that area. Meanwhile, passive sample selection was carried out on patients who came to health services. All participants were obtained by simple random sampling. This research obtained results from 1139 people suffering from malaria or a malaria prevalence of 30.7%.

Several characteristics of malaria sufferers assessed in this study include: Age, Gender, Clinical Symptoms, Diagnosis, Etiology, ABO Blood Type and Parasite Density. The age group most commonly found was 6-15 years old, namely 82.7%. Meanwhile, the majority of malaria sufferers were women, namely 53.6%. The clinical symptom assessed in this study was fever. Nearly a third of malaria sufferers experience clinical symptoms of fever, namely 61.7% (Table 1.)

Malaria diagnosis in this study was carried out using microscopic examination for 3 consecutive days. If plasmodium was found on the first day of the blood test, participants who have clinical symptoms of fever were declared to have clinical malaria. Meanwhile, those who did not have clinical symptoms of fever were referred to as asymptomatic malaria. However, if no plasmodium was found in the blood on the first day of examination, then the examination was continued until the third day. If plasmodium was found on the second or third day, all participants will be grouped as suffering from submicroscopic malaria, whether they had clinical symptoms of fever or none at all. The highest proportion of malaria sufferers was clinical malaria, namely 53.3% and the lowest proportion was submicroscopic malaria infection, namely 13.7%. This study also found 33.0% of asymptomatic malaria sufferers (Table 1).

Microscopy was also performed to identify the type of malaria parasite and parasite density in the blood of malaria patients. The diagnosis of malaria was determined by a minimum of two trained microscopists. If there was a difference of opinion between the two, then a further examination will be carried out by a third microscopist to determine the results of the examination based on the majority opinion. Plasmodium vivax was the most common parasite that causes malaria infections, namely 62.6%. Parasite density in this study was divided into two groups, with 840 parasites/μL of blood as the cut off value. The highest proportion of malaria sufferers was found in the parasite density group ≥ 840 parasites/μl blood, namely 56.2%. Meanwhile, blood group examination showed that the majority of malaria sufferers had blood type O, namely 42.9% (Table 1).

Table 1. Characteristic Of Malaria Patients

Characteristic	*n*	*%*
Age		
≤ 5 years	*167*	*14.7*
6 - 15 years	*942*	*82.7*
16 - 65 years	*30*	*2.6*
Gender		
Male	*528*	*46.4*
Female	*611*	*53.6*
Symptoms		
Fever (+)	*703*	*61.7*
Fever (-)	*436*	*38.3*
Diagnosis		
Clinical malaria	*607*	*53.3*
Asymptomatic malaria	*376*	*33.0*
Submicroscopic malaria	*156*	*13.7*
Etiology		
Plasmodium vivax	*713*	*62.6*
Plasmodium falciparum	*202*	*17.7*
Mixed	*224*	*19.7*
Blood Group		
O	*489*	*42.9*
A	*333*	*29.3*
B	*268*	*23.5*
AB	*49*	*4.3*
Parasite density		
<840	*499*	*43.8*
>=840	*640*	*56.2*

Overall the percentage of malaria patients with clinical symptoms of fever was the highest among all blood types. Fever is the most common clinical symptom in patients with blood type AB (71.4%) and the least common in patients with blood type B (59.3%). The most common clinical symptom found in malaria patients who have blood type AB was fever (71.4%) and the least common was found in blood type B (59.3%). Furthermore, among malaria patients without clinical symptoms such as fever the most common type is type B (40.7%) and the least common type is AB type (28.6%). Bivariate analysis of these two variables did not show significant differences in malaria patients ($p > 0.005$).

Vivax malaria occurs most often in those with blood group B (64.6%) and least often in those with blood group O (60.5%). Meanwhile, *Plasmodium falciparum* was the most common cause in blood type O (20.4%) and the least in blood group AB (14.35). The results of bivariate analysis stated that no significant relationship was found between these variables in malaria sufferers ($p > 0.05$).

Patients with blood type B (46.6%) had the highest proportion of parasite density < 840 parasites / µL of blood, whereas patients with blood type AB (38.8%) had the lowest. Meanwhile, the largest proportion of parasite densities ≥

840 parasites/µL of blood were sufferers with blood type AB (61.2%) and the lowest were sufferers with blood group B (53.4%). The variation in parasite density by blood group, however, did not differ substantially (p > 0.05) (Table 2).

Table 2. Statistical Bivariat Test Results

Characteristic	O		A		B		AB		p
	n	%	n	%	n	%	n	%	
Etiology									
Plasmodium vivax	296	60.5	214	64.3	173	64.6	30	61.2	
Plasmodium falciparum	100	20.4	48	14.4	47	17.5	7	14.3	0.353
Mixed	93	19.1	71	21.3	48	17.9	12	24.5	
Symptoms									
Fever (+)	295	60.3	214	64.3	159	59.3	35	71.4	0.271
Fever (-)	194	39.7	119	35.7	109	40.7	14	28.6	
Parasite density									
<840	214	43.8	141	42.3	125	46.6	19	38.8	0.644
≥840	275	56.2	192	57.7	143	53.4	30	61.2	

The highest hemoglobin levels were found in blood group AB (13,208 ± 0.226) and the lowest were blood group B (13,035 ± 0.709). Nonetheless, hemoglobin levels in malaria patients did not differ significantly depending on blood type (p > 0.05) in most cases. In the meantime, blood type B had the lowest parasite density (795.90 ± 344,031) and blood group O had the highest (851.45 ± 346,594). In common, there was no noteworthy contrast between parasite densities in all blood bunches, but between blood bunches O and B (p= 0.040) (Table 3).

Table 3. Comparison of hemoglobin concentration and parasite density

		Mean	SD	p
Hemoglobin [a]	O	13.090	0.529	0.216
	A	13.099	0.570	
	B	13.035	0.709	
	AB	13.208	0.226	
Parasite density [b]	O	851.45	346.594	0.186
	A	849.67	373.275	
	B	795.90	344.031	
	AB	829.39	393.851	

[a] One way anova. Post hoc LSD;
O vs A p=0.834 ; O vs B p=0.208; O vs AB p=0.174;
A vs B p=0.178; A vs AB p=0.217; B vs AB p=0.054

b One way anova. Post hoc LSD;
O vs A p=0.944 ; **O vs B p=0.040**; O vs AB p=0.675
A vs B p=0.066; A vs AB p=0.710; Bvs AB p=0.545

DISCUSSION

This study found that fever (61.7%) was still the dominant clinical symptom in malaria sufferers. This was in line with the finding of a higher proportion of clinical malaria (53.3%) compared to other types of malaria. This research also proved how important it is to carry out microscopic malaria screening, especially in people who have risk factors even though they do not have clinical symptoms of fever. This was proven by the discovery of 33% of asymptomatic malaria patients on the first day of examination and 38.5% of submicrocopic malaria sufferers who did not have clinical symptoms of fever on the second and third days of examination. The same thing must also be considered if the results of the examination on the first day were still negative, then patients who had risk factors still need to undergo examinations on the second and third days. This was proven by the discovery of 61.5% of submicroscopic malaria sufferers with clinical symptoms of fever on the second and third days of examination.

Plasmodium sp. is a parasite that lives most of its life in erythrocytes. The entry of malaria parasites into erythrocytes begins with the parasite attaching to receptors on the surface of the erythrocyte membrane. Research on the role of the erythrocyte surface during parasite invasion indicate the existence of specific receptor-ligand interactions between parasites and erythrocytes.[6]

ABO blood group antigens are glycoconjugate structures located on the surface of the erythrocyte membrane and are heritable.[9] The oligosaccharide structure consists of A, B and H antigens which determine the ABO blood group and function in cell physiological processes.[7] One of them is as an exogenous ligand receptor that allows microorganisms such as malaria parasites to attach before entering red blood cells.[8] Polymorphisms in the structure of glycoconjugates found in these blood groups greatly influence the host-pathogen relationship and result in differences in susceptibility between individuals. [9] Several studies have shown an association between *falciparum malaria* and ABO blood relatednessHumans who have blood type O have a lower risk of experiencing malaria infection compared to other blood types.[10,11]

This research showed that the majority of malaria sufferers had blood type O, followed by blood groups A, B and AB respectively. Many other studies have found similar results with more malaria patients having type O blood than other blood types.[12-17] However many studies have also found mixed results with blood type A having the highest amount and blood type having the lowest amount.[18-20] This difference in results was due to differences in the etiology of malaria and the presence of genetic mutations in red blood cells [21] as well as differences in climate and endemicity of a region.[4]

The majority of malaria patients obtained in this study were caused by *Plasmodium vivax*. This can also be associated with the relatively mild clinical manifestations of sufferers. Other studies also found the same results, namely that the clinical manifestations of *vivax malaria* patients were no worse than those of *falciparum malaria* patients.[22,23] The absence of significant differences in clinical symptoms with the plasmodium causing infection in this study was more due to the inhomogeneity of the research data, where *Plamodium vivax* was much more dominant than *Plasmodium falciparum*.

The clinical manifestations of *Plasmodium falciparum* infection were generally worse compared to other *plasmodium*. This was often associated with *Plasmodium falciparum* ability to attack red blood cells. Worsening of clinical manifestations was closely related to the

occurrence of rosseting events. Rosette formation occurs when some uninfected erythrocytes attach to erythrocytes that have been invaded by parasites. This binding process involves numerous parasitic ligands and erythrocyte receptors containing various proteoglycan, glycoproteins and carbohydrate fragments on the surface of erythrocytes. Laboratory studies have shown that *Plasmodium falciparum* expresses ligands on the surface of infected red blood cells allowing group A and group B carbohydrate antigens to attach to the blood until rosettes eventually form. Massive resetting events have the potential to cause vascular obstruction and endothelial inflammation. This condition was further worsened by the attachment of infected erythrocytes to receptors on the blood vessel endothelium, resulting in sequestration events.[24] This series of events can cause disruption of blood flow in small blood vessels.

Heterogeneity of ABO blood groups influenced parasite adhesion and invasion into erythrocytes.[24] The study found no significant difference in the blood groups of malaria patients. This result differs from many other studies which reported that malaria infections were more common in non-O blood groups than in O blood groups especially malaria caused by *Plasmodium falciparum.*[18,19,25] This was the basis for the difference in the results of this study from other studies.

The parasite density in this study was generally <1000 parasites/µL blood. Parasite density was often associated with disease severity. Some studies show that blood type O protected against severe malaria.[25] The results of this study prove that there was no significant difference between parasite density based on ABO blood group in the one-way ANOVA test ($p>0.05$). However, in further analysis using post hoc LSD, a significant difference was found between blood group O, which had the highest parasite density, and blood group B, which had the lowest parasite density ($p=0.040$). This finding differs from other studies which found that parasite density was lower for blood type O than for non-O blood types.[4,25] These differences in results occur due to differences in the etiology of malaria and the endemicity of the region.[25]

This relatively low parasite density was related to the hemoglobin levels found in this study, the majority of whom did not experience anemia. The results of this study prove that hemoglobin levels in malaria patients who have a low parasite density are not influenced by differences in the patient's blood type. The results differ from several other studies that found that people with type A blood were more likely to develop anemia than other blood types.[10,26,27] This difference in results occurs due to the etiology of malaria and regional endemicity.[25]

Plasmodium invasion of red blood cells was highly dependent on its adhesion ability to red blood cells. A person's susceptibility to malaria infection was related to red blood cell polymorphisms.[4] The rate of invasion of *Plasmodium falciparum* is very dependent on antigens in the ABO blood group as well as abnormalities in hemoglobin such as HbS, HbC, Thalassemia, G6PD and other hemoglobinopathies,[28-30] even increasing in patients without clinical symptoms.[31-33] Differences in results in several other studies were closely related to geographical location and endemicity of the region. Meanwhile, susceptibility to *Plasmodium vivax* was largely related to the Duffy blood group system.[9] All of these conditions have the potential to be risk factors for malaria.

The highest proportion of malaria sufferers in the study was caused by *Plasmodium vivax*. This profile was still in accordance with data on malaria sufferers

in the last 10 years in this study area. These results were also in accordance with the characteristics of vivax malaria which generally has relatively mild clinical manifestations in the form of fever. It was even found that there were sufferers of asymptomatic malaria and submicroscopic malaria. This study confirmed that there was no relationship between ABO blood group and malaria infection. This is because *Plasmodium vivax* dominates over *Plasmodium falciparum*. It can be seen that in areas of low malaria prevalence where *Plasmodium vivax* was the dominant species, blood groups based on the ABO system cannot be used as a predictor of malaria.

CONCLUSION

In low endemic areas where malaria was primarily caused by *Plasmodium vivax*, blood type based on the ABO system is not a risk factor for malaria infectionThe discovery of asymptomatic malaria sufferers suggests the need to change malaria screening methods that do not only examine patients who have fever.

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