Association Between ABO Blood Groups and Malaria Severity in a Regional Referral Hospital in Jayapura Papua, Indonesia

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ABSTRACT

Background: *Malaria infection has caused a significant morbidity and mortality, notably in high-risk groups. Some evidence showed that ABO blood types might associate with malaria severity. This study aimed to determine the relationship between blood types and malaria severity in Papua, as Papua is a malaria-endemic area.* ***Methods:*** *A cross-sectional study was conducted in a regional referral hospital in Jayapura, Indonesia. Diagnosis of malaria was determined using World Health Organization criteria and classified into severe and uncomplicated malaria. Blood types were classified into O and non-O groups.* ***Results:*** *Out of 210 patients, 84 (40%) and 126 (60%) patients had non-O and O blood types, respectively. Severe malaria was more prevalent in non-O compared to O blood type (16.7% vs. 9.5%; the prevalence ratio (PR) was 2.4; 95% CI 1.06-6.42; p value 0.032). Amongst non-O groups, group B blood type demonstrated the highest incidence of severe malaria (p value 0.038; 95% CI: 1.06-6.42).* ***Conclusion:*** *There is an association between ABO blood group and the severity of malaria in Papua. Severe malaria was found more in non-O blood types, especially type B blood group.*

Keywords*: ABO blood type, infection, malaria, severity of malaria.*

INTRODUCTION

Malaria is an infectious disease caused by Plasmodium species and has infected hundreds of millions of population worldwide with a high mortality rate. Globally, there were an estimated 247 million malaria cases in 2021 in 84 malaria endemic countries. Malaria case incidence reduced from 82 in 2000 to 57 in 2019, before increasing to 59 in 2020. There was no change in case incidence between 2020 and 2021.[1] According to WHO's latest *World malaria report*, there were an estimated 241 million malaria cases and 627 000 malaria deaths worldwide in 2020. Indonesia is one of nine malaria-endemic countries in the South-East Asia region. In 2010 positive cases of malaria in Indonesia reached 466.7 thousand, while in 2020 positive cases decreased to 235.7 thousand.[2] In

Indonesia Malaria cases occur in all age groups, the most cases occur in the productive age group, namely 15-64 years old.[3] Papua is the most malaria-endemic area in Indonesia with the highest incidence and prevalence with the Annual Parasite Index (API) 80.05 per 1000 population.[3] This indicator is obtained by calculating the proportion between patients malaria positive for at-risk populations in certain areas. Indonesia successfully suppresses API to less than 1 from 2015 to 2020. Nevertheless, in 2021 API increased to 1.1 per 1,000 risk population.[3]

Severe malaria is associated with high mortality rate. Pathogenesis of severe malaria involves the parasite, host and social geographic factors.[4] One of the considerable host factors is the ABO blood type. ABO blood phenotypes are derived genetically and may differ between

individuals and ethnicities. The presence or absence of A and B antigen on the surface of erythrocytes is considered to be able to affect the severity of malaria.[5] Degarege et al[4] found an increased odds of severe P. falciparum infection among individuals with blood group A, B, AB or non-O compared with blood group O. Several studies such as Deepa et al.[6] found association between ABO blood type and severity of malaria. Contradictory, such association was not identified by Thakur et al.[7] in India and Fowkes et al.[8] in Papua New Guinea.Those differences in results might be affected by demographical characteristics and geography, which influence the parasite transmission, immunological, and gene expression. This has raised the question on the association between the incident of severe malaria and ABO blood type in Papua Indonesia.

METHODS

This is a cross-sectional study conducted in a regional referral hospital Dok II Jayapura Papua Indonesia from September 2016 to November 2016. Subjects were collected consecutively. Written consent was retrieved from the subjects prior to data collection. The sample size of this study is calculated by using this formula:

$$n = \frac{(Z\alpha\sqrt{2PQ} + Z\beta\sqrt{2P1Q1 + P2Q2})^2}{(P1 - P2)^2}$$

The incident effect on the group with risk factor (P1) is estimated around 0.39. After the calculation, the P2 is 0.21 with the level significance Zα = 1.96 and Zβ = 80%. Therefore, the sample needed for this study is 202 subjects.

The ABO blood type examination was performed using the standard agglutination method. The blood types were classified into O and non-O types. The parasite was examined with light microscope from thin and thick blood smears, while the number of parasites was reported per 200 leukocytes. Hemoglobin level, blood sugar, bilirubin, and creatinine levels were also reported. Malaria's clinical manifestation was categorized into mild and severe malaria according to WHO criteria. The data were analyzed using SPSS version 23 and reported based on bivariate analysis (chi-square or Fisher exact test) and prevalence ratio with confidence intervals. The study was approved by the ethical committee of the Faculty of Medicine, Universitas Indonesia, Jakarta (ethical clearance number 767/UN2.F1/ETIK/2016).

RESULTS

Two hundred and twenty-two subjects were recruited for this study. Of those, 10 were excluded because they had significant comorbidities, such as renal failure, heart failure, pregnancy, Parkinson disease, and infected by Plasmodium malariae (as Plasmodium malariae infection doesn't not lead to severe infection).

The demographic characteristics of the subjects, the blood types, and plasmodium infection data were presented in **Table 1** and **Table 2**. Most of the subjects were Papuan, male, age under 50 years old, had no comorbidities, and had suffered from malaria at least three times. Most of the subjects lived in Jayapura. The parasite density of O blood type and non O blood type can be seen in **Table 3**. We also tested for malarial density index compared between O blood type and non-O blood type with Kruskal-Wallis test. The result of Kruskal-Wallis test can be seen in **Table 4**.

We did not identify significant differences in proportions between blood types in each species of plasmodium infection (*P. Falciparum* (p value 0.158, PR 1.53, 95% CI 0.87-2.73), *P. vivax* (p

Table 1. Characteristics of Subjects.

O Blood Type		Non-O Blood Type	
Characteristics	**n (%)**	**Characteristics**	**n (%)**
Age		Age	
• 17-40 years	97 (76,3%)	• 17-40 years	72 (85,7%)
• 41-59 years	24 (18,9%)	• 41-59 years	11 (13,1%)
• > 60 years	5 (4.8%)	• >60 years	1 (3,2%)
Sex		Sex	
• Male	82 (61,1%)	• Male	51 (60,7%)
• Female	44 (38,9%)	• Female	33 (39,3%)
Geographical origin		Geographical Origin	
• Papua	100 (79,4%)	• Papua	63 (75%)
• Non-Papua	26 (20,6%)	• Non-Papua	21 (25%)
Occupation		Occupation	
• Civil servant	13 (10,3%)113	• Civil Servant	7 (8,3%)
• Non-civil servant	(89,7%)	• Non-Civil Servant	77 (91,7%)
Level of education		Level of Education	
• Not attending school	6 (4,8%)	• Not attending school	2 (2,5%)
• Elementary school	11 (8,7%)	• Elementary School	6 (7,1%)
• High school	96 (76,2%)	• High School	68 (80,9%)
• College	13 (10,3%)	• College	8 (9,5%)
Financing		Financing	
• Covered by the Government	123 (97,8%)	• Covered by the Government	81 (96,5%)
• Self-funding	3 (2,3%)	• Self-funding	3 (3,5%)
Domicile		Domicile	
• Jayapura City	110 (87,3%)	• Jayapura City	78 (92,8%)
• Outside Jayapura City	16 (12,4%)	• Outside Jayapura City	6 (7,2%)
History of Malaria		History of Malaria	
• Present	119 (94,5%)	• Present	81 (96,5%)
• Absent	7 (5,5%)	• Absent	3 (3,5%)
Median count of malaria parasites	6748 (319-220.000) parasite/microliter		
Hemoglobin	12.6 g/dl		
Leukocytes	7.143/mm^3		
Platelets	85,000 (32-206.960) /mm^3		

Table 2. Prevalence of Plasmodium Infection on Each Blood Type.

Blood Type	**Plasmodium**			**Total n (%)**
	Pf (%)	**pv (%)**	**pf and pv (%)**	
Non O (A+B+AB)	47 (45.2%)	33 (34.4%)	4 (40%)	84 (40%)
A	24 (23%)	18 (18.8%)	3 (30%)	45 (21.4%)
B	20 (19.2%)	12 (12.5%)	1 (10%)	33 (15.7%)
AB	3 (3%)	3 (3.1%)	0 (0%)	6 (2.9%)
O	57 (54.8%)	63 (65.6%)	6 (60%)	126 (60%)
Total	104 (49.5%)	96 (45.7%)	10 (4.8%)	210 (100%)

pf : Plasmodium falciparum, pv : Plasmodium vivax, pf and pv : mixed

Table 3. Parasite Density of Each Blood Type.

Blood Type	**Parasite Density**				**Total n (%)**
	+1	**+2**	**+3**	**+4**	
Non O (A+B+AB)	19 (46%)	13 (41,9%)	15 (27,3%)	37 (44,5%)	84 (40%)
A	9 (21,9%)	8 (25,8%)	10 (18,2%)	18 (21,7%)	45 (21,4%)
B	7 (17,1%)	4 (12,9%)	5 (9,1%)	17 (20,4%)	33 (15,7%)
AB	3 (7%)	1 3,2%)	0 (0%)	2 (3,4%)	6 (2,9%)
O	22 (54%)	18 (58,1%)	40 (72,7%)	46 (55,5%)	126 (60%)
Total	41 (19,5%)	31 (14,7%)	55 (26,1%)	83 (39,7%)	210 (100%)

Table 4. Result of Kruskal-Wallis Test.

Factors	Kruskal-Wallis H	Degree of Freedom	p-value
Malaria Density Index	5.276	4	0.260

value 0.25, PR 1.44, 95% CI 0.8-2.54) and mixed (p-value 1.00, PR 1.08 95% CI 0.31-3.82). The prevalence of the severity of malaria in each blood type can be seen in **Table 5**.

Of 210 subjects, 40% had non-O blood type, and 60% had O blood type. The number of severe malaria cases in the non-O group was 14 (16.7%) and 12 (9.5%) in the O group. These difference in proportion was statistically significant (p value 0.032; prevalence ratio 2.4 (95% CI 1.06- 6.42). Amongst non-O group, B blood type demonstrated the highest proportion of severe malaria cases (28.2%; p value 0.038 95% CI (1.06-6.42). The prevalence ratio of severe malaria in non-Papuan was higher than in Papuan, but the difference between those groups was not statistically significant (PR 3.8 (95% CI 0.84 to 17.9) and (PR 1.83 95% CI (0.56 to 5.9; p = 0.143 and p = 0.356)).

DISCUSSION

Demographic Characteristics, ABO Blood Group, and Plasmodium

In this study, we found that malaria-infected patients had higher proportion in O blood type. This result was similar with those in other endemic regions, such as Kenya, Sudan, and Somalia (the proportion of O blood type were 60%, 62%, and 60% respectively).[9] In previous meta-analysis study, subjects in malaria endemic regions with O blood type were predominantly found with uncomplicated malaria, which indicates of its protective property in plasmodium infection, especially to P. falciparum.[10] Besides that, recent studies showed that blood group O can protect against severe malaria by reducing rosette formation, whereas the others could not.[11-14]

The most prevalent plasmodium infection in this study was *P. falciparum* (50.48%), followed by P. vivax (44.3%) and co-infection of both plasmodium (5.2%). This study found no significant differences in the types of plasmodium infection between blood type-O and non-O.

The Relationship of ABO Blood Type with the Severity of Malaria

Since the discovery of ABO blood type in 1900, various study investigated its correlation with malaria clinical outcome. Rubasckin and Leiserman in 1929 reported the significant correlation between malaria out come and ABO blood type.[5] Studies by Panda et al.[10] In India reported severe malaria were more frequent in non-O-blood type with *P. falciparum* infection compared to the other Plasmodium. However, some studies reported no such correlation. Degarege et al.[4] found that individuals with blood group A, B, and AB are more susceptible to severe *P. falciparum* infection and blood group O has a protective effect. Our study shows higher incidence of severe malaria in subjects with non-O blood type compared with those with O blood type. Amongst non-O blood type, B blood type showed the highest severe malaria

Table 5. Association between ABO Blood Group and Malaria Severity.

Blood Type	Malaria Infction				Total	PR (95% CI)	p Value
	Severe Malaria		Uncomplicated Malaria				
	N	%	n	%			
Non O (A+B+AB)	14	53.8	70	38	84	2.4 (1.06-6.42)	0,032
A	6	23	38	20.7	45	2.1 (0.71-6.34)	0.213
B	6	23	24	13	33	3.3 (1.09-10.3)	0.038
AB	1	7.8	5	4.3	6	2.6 (0.28-25.5)	0.373
O	12	46,2	114	62	126	1 Ref	
Total	26	12.4	184	87.6	210		

incidence. Furthermore, our study shows no significant correlation between ABO blood type and parasite density, which similar to previous study by Fowkes, et al.[8] In Papua New Guinea. Therefore, the different incidence of malaria severity between blood groups might not be related to the parasite density.

There are several explanations for the association between ABO blood types and severity of malaria. Firstly, the existence of antigens/glycoproteins A and B on erythrocytes surface affect size, strength, and frequency of the rosette formation. Infected erythrocytes express proteins, such as PfEMP-1, HSP-1 and rosetin, which form rosette binding to antigen A and B receptors, Complement Receptor-1 CD35, Heparan Sulfate-Like GAG, and CD36 on the surface of uninfected erythrocytes. In addition to forming rosette, PfEMP-1 also performs adhesion on the blood endothel through CD36 receptor, Intracellular Adhesion Molecule-1 (ICAM-I), P-Selection, Thrombospondin, Endothelial Leucocyte Adhesion Molecule (ECAM), Vascular Cell Adhesion Molecule (VCAM), Heparan Sulfate, E-selectin, and other receptors. Those earlier receptor interactions will enhance the adhesion process and rosette formation into giant rosette.[13] Besides that, Determination of the carbohydrate structure found in glycophorin A or B, including sialic acid and galactose, is an essential requirement for the entry of Plasmodium falciparum merozoites into human red blood cells. Sialic acid is common in several pathways that use glycophorins as ligands and is also associated with ABO phenotypes. Sialic acid is one of the key molecules for parasite attachment, and variations in malaria susceptibility between humans and chimpanzees are thought to be related to genetic changes in these molecules.The sialic acid molecule is found in glycophorin. In areas where Plasmodium falciparum malaria is particularly endemic, it is known that many red blood cell polymorphisms associated with exposure to severe malaria have been actively selected.[15] Barragan et al.[11] study confirmed that the strongest rosette was formed in blood type A. In other studies Degarege A et al.[4] which experimented converting blood type A into blood type O enzymatically, the rosette formation evidently decreased.

Secondly, in O blood type, the phagocytosis of erythrocyte schizont by monocytes and macrophages was more effective. This was due to the increase of pro-degradation product deposition, i.e. hemicrom and hight moleculer weight band 3.[5] Thirdly, blood type O may induce MSP2 of specific IgG, IgG1, IgG2 and IgG3, which eventually decreases the occurrence of severe malaria (p <0.001).[16]

Thirdly, the protective role of blood group O has intricate mechanisms. Individuals with blood group O may be selecetively advantaged from the co-existence of anti-A and anti-B antibodies shared by microorganisms. Diversity of surface glycan molecules in red blood cells and ligands such as merozoite surface protein 1, apical membrane antigen 1 and erythrocyte-binding antigen in Plasmodium may also lead to different host susceptibility.[16-20]

Association of ABO Blood Type with Severity of Malaria by Geographical Origin

Geographical factor had been one of the most considerable factors for malaria transmission, hence the term endemicity. In this study, we define the term non-Papuans to refer to the population who were not originally from Papua yet had lived in the region for at least a year. Based on the analysis associating ABO blood group, malaria severity and ethnicity, it is seen that non-O groups are carrying higher risk of severe malaria, especially for non-Papuans, (PR 3.8 [0.84-17.9], p-value 0.143) compared to O groups. Albeit not statistically significant, we believe that this number may be clinically important seeing that in non-Papua group, more than one-third of non-O had severe malaria whereas in Papua group, almost 10% of non-O had severe malaria. Yeda et al.[21] had demonstrated the association between ABO blood type, malaria severity and the possible association of endemicity as a considerable factor affecting malaria severity. Yeda et al.[21] also showed that those living in malaria-endemic zones may present higher parasite densities compared to those living in malaria-epidemic zones, although parasite density does not always correlate with disease severity. Further analysis showed that individuals from endemic zones demonstrated

high parasitemia with higher number seen in blood groups A and B than individuals of blood group O. This finding may happen due to the immunogenetic difference (innate immunity and acquired immunity). Papuans have been exposed to malaria since childhood, whilst not all non-Papuans have been exposed to malaria. The discrepancy of exposure to infection may cause the different immune response in native population.[18, 22-24] However, we find that albeit the geographical differences, non-O blood type group showed higher proportion of severe malaria compared to O blood type.

Cause and Effect Relationship Based on Hills' Criteria

We estimate the cause-and-effect relationships based on Hills' criteria found in this study to observe a causal relationship between ABO blood group and malaria severity. Firstly, there is a temporal relationship that ABO blood group (independent variable) preceded the plasmodium infection and the occurrence of severe malaria (dependent variable). Secondly, prevalence Ratio (PR) of severe malaria between type O and non-O blood group was 2.4 (95% CI 1.06 -6.42) and was statistically significant (p = 0.032), which aligns with previous studies. The result of this study is consistent with previous studies conducted by Deepa et al. [6] Africa Studies conducted by Barragan et al.[11] also reported that rosette formation is easier, bigger, and stronger among those with non-O blood group. Baragan et al. also found that if people with type A blood group were given α-N-acetyl-galactosaminidase, consequently depleting its antigen from its terminal α-N-acetyl-galactosamine, shows a drastic decrease formation of rosettes in type A blood group. The correlation of ABO blood group with malaria severity may also be demonstrated through this study. The studies currently available also supports the correlation of antigens/glycoproteins A and B in erythrocyte surface may increase the adherence, rossette formation, and sequestration.[7] Moreover, there is an increased clearance mechanism by the immune system towards the infected erythrocyte with non antigens/glycoproteins A and B. Type O blood group may induce IgG, IgG1, IgG2, and IgG3 which are specific against MSP2.[17,20-23]

CONCLUSION

To conclude, we found that patients with non-O blood type had more incident of severe malaria compared to those with O blood type, especially those with B blood type.

CONFLICTS OF INTEREST

The authors affirm no conflict of interest in this study.

ACKNOWLEDGMENTS

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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