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# *PLASMODIUM FALCIPARUM* INFECTIONS AMONG INDIVIDUALS WITH DIFFERENT ABO BLOOD GROUPS ATTENDING KIPKELION AND FORT-TENAN HOSPITALS IN KERICHO COUNTY, KENYA

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## ABSTRACT

**Objectives:** The study was designed: (i) to determine the prevalence level of *Plasmodium falciparum* infections among individuals with different ABO and Rhesus blood group phenotypes; (ii) to determine the relationship between levels of *P. falciparum* parasitaemia and ABO blood groups; (iii) to determine the relationship between levels of parasitaemia and age of patient; and (iv) to determine how *P. falciparum* infections compare in relation to sex. **Methods**: Blood samples from patients with clinical symptoms of malaria were microscopically analyzed for confirmation of *P. falciparum* parasites. Both thick and thin blood films were prepared from the blood samples of each patient. The remaining portion of blood was used for determination of blood group using commercial antisera –A, B and D and observing for agglutination. **Results:** Microscopic examination of blood slides showed that 169 patients were malaria positive and 137 were negative out of the total 306 patients sampled. *P. falciparum* prevalence level in Kipkelion west district were 32.5% for blood group A+, 22.5% for blood group B+, 20.1% for blood group O+ while O- individuals had no infections by microscopy. *P. falciparum* infection had significant and positive association with blood types A+ ($\chi^2$=10.38, d.f=1, $p$=0.001), B+ ($\chi^2$=2.68, d.f=1, $p$=0.049) and AB+ ($\chi^2$=1.98, d.f=1, $p$=0.003). Regression analysis showed a strong positive relationship between age category and parasitaemia levels ($r^2$ = 0.945, F=481.7, d.f=6, $p$=0.001). Chi-square revealed significant difference between mean *P. falciparum* parasitaemia in blood groups A+ ($\chi^2$=13.39, d.f=1, $p$=0.001), B+ ($\chi^2$=6.78, d.f=1, $p$=0.001) and AB+ ($\chi^2$=2.95, d.f=1, $p$=0.086). Chi-square revealed a positive association between Rhesus positive individuals and *P. falciparum* infections ($\chi^2$ = 80.6, df= 2, $p$= 0.0001) while Rhesus negative individuals showed lack of association with *P. falciparum* infections ($\chi^2$ = 445.2, df= 2, $p$= 0.067). **Conclusion:** Individual's blood group can predispose or confer resistance to *P. falciparum* infection as evidenced in the results of the current study which suggest that individuals with blood groups A+, B+ and AB+ are more susceptible to *P. falciparum* infections than individuals with blood groups A-, B-, O- and O+. Also individual's age category is a risk factor to acquiring *P. falciparum* infection.

**KEYWORDS**: *Plasmodium falciparum*, parasitaemia, EDTA.

## INTRODUCTION

Malaria is a protozoan disease caused by various etiological agents which include *Plasmodium falciparum*, *Plasmodium malariae*, *Plasmodium ovale*, *Plasmodium vivax* and *Plasmodium knowlesi*. *P. falciparum* accounts for about 90% deaths due to malaria.[1] The World Health Organization (WHO) estimated that malaria causes 250 million cases of fever annually.[2] The disease is mainly endemic in regions around the equator, including sub-Saharan Africa where 85–90% of malaria cases are fatal. There is increasing evidence that *P. falciparum* malaria is influenced by ABO blood type of an individual but the extent of association is not fully established.[3] Some investigators have expressed the opinion that genotype is a factor in susceptibility to *Plasmodium* species infection in humans[4,5.6] while other investigators reported absence of significant association between *P. falciparum* and ABO antigens.[7,8,9] Despite the above researchers, there is however, still lack of consensus on possible association between ABO blood groups and *P. falciparum* infections.[4,5,6] This might be due to limited data on the association between malaria and red blood cell ABO antigens.[10] It is for these reasons that this study was designed to investigate whether there is an association between individual's ABO blood group and *P. falciparum* infections.

## MATERIALS AND METHOD

**Study area:** The study was done at Kipkelion and Fort-tenan Sub-district hospitals located in Kipkelion West district in Kericho County between the months of

November 2012- January 2013 and March-August 2013.

**Ethical clearance:** All study participants or guardians were informed of the aim of the study and requested to fill a written consent form. Data obtained from patients was treated with confidentiality. Research clearance was obtained from Kenyatta University Graduate School and administration of Fort tenan and Kipkelion Sub-district Hospitals.

**Subject selection:** The study participants comprised of 306 patients between the age 1–70 years and must have been reffered to the laboratory for malaria test. The patients presented clinical signs and symptoms of malaria such as fever, headaches, loss of appetite, joint pains, vomiting, nausea and malaise. They were confirmed to be infected with the *P. falciparium* malarial parasite by microscopic examination of Giemsa stained thin blood slides.

**Blood collection and staining:** Blood samples from the patients were collected following the normal routine aseptic procedure into an EDTA-anti coagulant tube using a 5ml syringe and needle set. Two drops of blood were placed separately on each half of a clean glass slide then thick and thin films prepared on the other half of the same slide and allowed to air dry. The remaining part of the blood was used for determination of ABO and Rh blood group type of each patient. Thin blood film was fixed with methanol for one minute. The slides were then immersed in a staining trough and placed on a staining rack. Giemsa stain diluted with phosphate buffer of pH 7.2 in the ratio 1:10 was used to stain the blood film for 10 minutes. Excess stain was washed off the slides using the phosphate buffer. The back of the slides were wiped dry with paper towel and placed on a draining rack for the blood film to air-dry.

**Examination of samples:** Microscopic examination was done using oil immersion under x100 objective lens magnification. Thin blood film was used for *Plasmodium* species identification. Thick films for each patient were examined to determine the parasite species and the stages that were present. For positive blood films tally counts were done for parasites and leukocytes simultaneously the number of parasites relative to leukocyte counts were converted to parasites per micro liter using the following formulae:

Parasitaemia / micro liter of blood ($\mu l^{-1}$) =

Number of parasites/ Number of leukocytes× 8000

Where, 8000 is the mean number of leukocytes/µl of blood.[11]

**Determination of ABO and Rh blood groups:** Two clean grease free microscope slides were used in determination of ABO and Rh blood group for each patient. Using a glass marking pencil, a line was drawn across the centre of the slide to divide it into two equal portions. Each portion of the two slides were labeled 1,2,3 and 4 in which two drops of antisera-A, B, saline and antiserum-D were placed on the four portions respectively. Two drops of well mixed 5% patient's blood in saline were added onto each portion and using an applicators stick, separate for each slide, the cells and the antisera were mixed well and after about 2 minutes observation was done to check for agglutination. The part marked 3 (blood + normal saline) acted as a control. In this portion agglutination did not occurred but if it occurred the whole procedure was repeated. In portion 4 (blood + anti-D), if agglutination occurred the blood was Rhesus positive and if there was no agglutination it was Rhesus negative.

**Statistical analysis:** Comparison of levels of parasitaemia amongst individuals of different ABO and Rhesus blood groups was statistically analyzed using Chi-square ($\chi^2$). The relationship between parasitaemia levels with respect to age and sex were analyzed using regression analysis. $p<0.05$ was considered significant.

## RESULTS

Among the 306 patients sampled, it was found that *P. falciparum* infection had significant and positive association with blood types A+ ($\chi2=10.38$, df=1, p=0.001), B+ ($\chi2=2.68$, df=1, p=0.049) and AB+ ($\chi2=1.98$, df=1, p=0.003) with the highest proportion observed among individuals with blood group A+ 55 (32.5%) followed by those with blood group B+ 38 (22.5%) then O+ 34 (20.1%) while O- individuals had no infections by microscopy (Table 1).

**Table 1: *Plasmodium falciparum* infection rates in individuals with different ABO and Rhesus blood groups.**

| Blood group | Infected (%) | Uninfected (%) | Total | $\chi2$ | *p* value |
|---|---|---|---|---|---|
| **A+** | 55 (32.5) | 26 (19.0) | 81 (26.5) | 10.38 | 0.001 |
| **B+** | 38 (22.5) | 25 (18.2) | 63 (20.5) | 2.68 | 0.049 |
| O+ | 34 (20.1) | 55 (40.1) | 89 (29.0) | 1.53 | 0.216 |
| **AB+** | 25 (14.8) | 16 (11.7) | 41 (13.3) | 1.98 | 0.003 |
| O- | 0 (0) | 2 (1.5) | 2 (1.0) | - | - |
| A- | 8 (4.7) | 6 (4.4) | 14 (4.5) | 0.29 | 0.593 |
| B- | 9 (5.3) | 7 (5.1) | 16 (5.2) | 0.06 | 0.808 |
| Total | 169 (100) | 137 (100) | 306 (100) | | |

The blood groups A+, B+ and AB+ were significant different ($p<0.05$). Figures in parentheses are percentages.

Young children aged 0-9 had high levels of mean parasitaemia (4482.2 per µl of blood) followed by individuals of age category 60-69 year (4289.3 per µl of blood (Figure 1). Regression analysis revealed a positive

association between mean parasitaemia levels and age groups ($r^2$=0.945, F=482.7, d.f=6, p=0.0000). This suggests that the individual's age category is a risk factor to acquiring complicated cases of *P. falciparum* infection. The age categories 0-9 (r=-1.7, d.f=6, p= 0.000) and 60-69 (r=-0.29, d.f=6, p=0.000) years showed significant association with *P. falciparum* malaria infection. Among the infected individuals, a high proportion (90.5%) were Rhesus positive while Rhesus negative individuals were 9.5% (Table 2). Chi-square revealed a positive association between rhesus positive individuals and *P. falciparum* infections ($\chi^2$ = 80.6, df= 2, *p*= 0.0001) whereas no association where observed among rhesus negative individuals ($\chi^2$ = 445.2, df= 2, p= 0.067). Therefore, this study suggests that rhesus positive individuals are more likely to be infected with *P. falciparum* than rhesus negative individuals living on the same malaria endemic area.

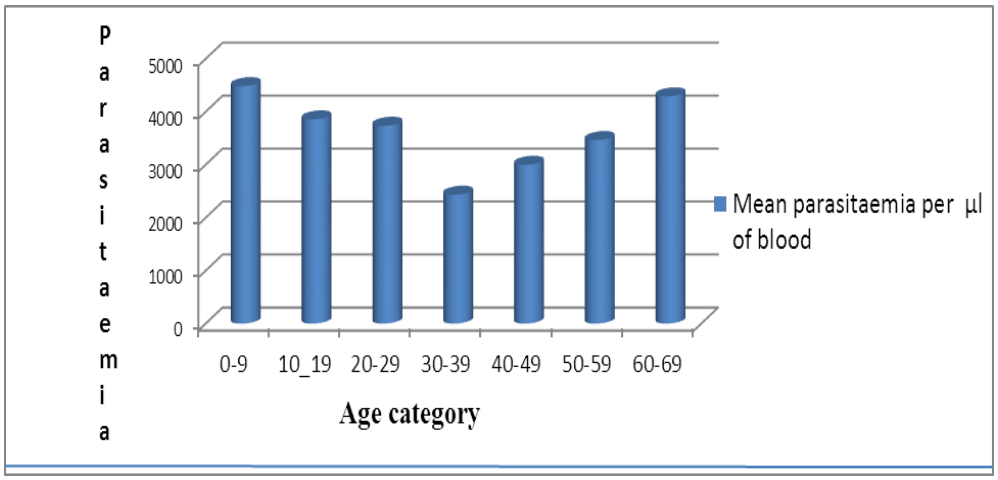


**Figure 1: Levels of parasitaemia in individuals of different age groups.**

**Table 2: Rhesus factor and *Plasmodium falciparum* infections.**

| | Infected (%) | Uninfected (%) | Total | $\chi^2$ | *p* value |
|---|---|---|---|---|---|
| **Rhesus positive** | 153 (90.5%) | 123 (89.8%) | 276 | 80.6 | 0.0001 |
| **Rhesus negative** | 16 (9.5%) | 14 (10.2%) | 30 | 445.2 | 0.067 |
| **Total** | 169 (100%) | 137 (100%) | 306 | | |

Rh positive individuals showed significant association with *P. falciparum* infection (p<0.05).
Figures in parentheses are percentages.

## DISCUSSION

The results of the current study showed statically significant and positive association between *P. falciparum* infection and blood types A+ ($\chi^2$=10.38, p=0.001), B+ ($\chi^2$=2.68, p=0.049) and AB+ ($\chi^2$=1.98, p=0.003) suggesting that individuals with these blood types were more susceptible to *P. falciparum* infections than individuals with blood groups A-, B-, O- and O+. This implies that individual's blood group can predispose or confer resistance to *P. falciparum* infection. Rosetting has been established as a *P. falciparum* virulent factor, the expression of which is modified by a variety of host factors which include the ABO and Rh blood types.[12] It is reported that blood type A is the most often affected by severe malaria and rosettes formed in blood types A and B are larger, tighter and stronger than those formed in persons with blood type O.[13] Type O seems to have a large preventative effect in severe malaria cases. It does not protect people from initial infection, according to these comparisons, but it seems to reduce the disease severity by reduction in rosetting. It has been found that type O people lack the functional trisaccharide antigen, and therefore they have less rosetting when infected by *P. falciparum*.[14] Results from the current study concur with earlier studies done among febrile outpatients who sought medical attention at Dore Bafeno Health Center, Southern Ethiopia, where it was reported that individuals with blood groups A, B and AB were more susceptible to *P. falciparum* infection as compared with those of blood group O.[15]

It was observed that young children aged 0-9 had high levels of mean parasitaemia (4482.2 per µl of blood) followed by individuals of age category 60-69 year. On statistical analysis it was realized that parasitaemia levels had significant association with *P. falciparum* infection ($r^2$=0.945, F=481.7, d.f=6, p=0.000) suggesting that individuals belonging to the two age categories are at risk of acquiring P. falciparum malaria than the other age categories. Possible explanation for the significant association of age group 0-9 years is because the immune system of infants is not yet fully developed, while in children under five years they have not yet developed effective resistance to the disease. As the children grow and are bitten repeatedly by an infected mosquito they gradually build up resistance to the malaria parasites.[16] The old people of age 60-69 were at risk since 14.2% of the sampled populations were positive and this could be attributed to their reduced immunity due to old age.

The results from the current study suggest that Rhesus positive individuals ($\chi^2$ = 80.6, df= 1, *p*= 0.0001) are more likely to be infected with *P. falciparum* infections than Rhesus negative individuals ($\chi^2$ = 445.2, df= 1, *p*= 0.067) living on the same malaria endemic area. In earlier studies done in Portuchuelo, Brazil, no significant associations were observed between Rhesus factor and *P. falciparum* infections ($\chi^2$=8.943, df=7, *P*=0.257). Since Rh blood group system consists of different antigens (D, C, c, E and e), the researchers were further prompted to retest the association using the other phenotypes and they found that individuals with EE antigens exhibited a higher number of malaria episodes than those with ee antigens.[17]

## CONCLUSION

*P. falciparum* prevalence level in Kipkelion west district were 32.5 % for blood group A+, 22.5 % for blood group B+, 20.1 % for blood group O+ while O- individuals had no infections by microscopy. Individual's blood group can predispose or confer resistance to *P. falciparum* infection as evidenced in the results of the current study which suggest that individuals with blood groups A+, B+ and AB+ are more susceptible to *P. falciparum* infections than individuals with blood groups A-, B-, O- and O+. Blood group O seems to confer to a certain degree of protection against severe cases of malaria. Individual's age category is a risk factor to acquiring *P. falciparum* infection. The results of the current study indicated that individuals of age groups 0-9 and 60-69 years were more predisposed to *P. falciparum* infections than age groups 10-19, 20-29, 30-39, 40-49 and 50-59 years. Their parasitaemia levels were also higher than the later age categories. Males were more predisposed to *P. falciparum* infections than females. Chi-square revealed a statistically significant difference between mean parasitaemia levels for males and no significant difference for females suggesting that males were more susceptible and tended to develop more severe cases of *P. falciparum* infections than females.

## RECOMMENDATIONS

Individuals with blood groups A+, B+ and AB+ should take prophylactic drugs against malaria during on-set of short and long rainy seasons and also when traveling to malaria endemic regions. More awareness about blood group should be created among residents of Kipkelion West District as this would go a long way in reducing morbidity and mortality due to malaria and perhaps other blood diseases. Apart from the young children and pregnant women, the government should include the old people (60 and above years) and males in the current control strategy for malaria in order to reduce malaria transmission. These individuals are considered to be the most susceptible according to results and conclusions from the current study. Since there is high malaria transmission in the months of May, June and July members of the public should strictly adhere to the various preventive measures so as to reduce malaria transmission in those months.

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