Do Blood group and Sickle cell trait protect against placental malaria?

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Abstract. Blood group O is reported to confer some degree of protection from severe malaria in endemic setting. This protection is believed to be due to reduced and smaller rosette formation in people of blood group O which can easily be cleared by the host immune system. Also, sickle cell trait (HbAS) is reported to disrupt the adhesion of infected erythrocytes to microvascular endothelial walls, which could protect pregnant women from placental malaria. We determined the association between HbAS and ABO blood group, and placental malaria amongst pregnant women of all parities. The study enrolled 221 pregnant women. Peripheral blood samples were taken for malaria smears, ABO blood grouping and haemoglobin (Hb) electrophoresis. A structured questionnaire was used to age, bed net usage, and the number of Sulphadoxine-pyrimethamine (SP) doses taken by a pregnant woman. Two hundred and twenty-one (221) pregnant women were enrolled and out of this number, 110 (49.8%) were primiparae and 111 (50.2%) multiparae, with a mean age of 23.7±5.2. Placental malaria (PM) prevalence by PCR detection was 19.4% (43/221). Of those who were malaria positive 58.1% (25/43) were primiparae. Primiparae who are of blood group O were more susceptible to PM [P=0.04, (OR); 2.85, 95% (Cl), 1.12-9.01]. But sickle cell trait did not reduce the prevalence of PM [P=0.84 (OR); 0.92, 95% (Cl), 0.43-1.99]. Non-blood group O primiparae women were protected against placental malaria. This could be why some primiparae women are protected from PM, just like multiparae women.

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Introduction

Malaria during pregnancy can be detrimental to the pregnant woman and the unborn child. It may result in maternal death, low birth weight or still births (1-3).

There are many factors that have been linked to this poor outcome of pregnancy, which include host factors (4) and *P.* falciparum factors (5). One of the major complication of malaria in pregnancy (MIP) is when parasitized erythrocyte sequester in the placenta (6,7). These *Plasmodium falciparum* infected erythrocyte sequester in the placenta using a unique protein called VAR2CSA protein on their surfaces (8-10). These sequestrations alters placenta function (11,12) by impeding maternal-foetal exchange of nutrients, often resulting in adverse pregnancy outcomes (10,13).

Some hemoglobinopathies contribute to reduced host's susceptibility to malaria (14,15), specifically, sickle cell trait (HbAS, HbSC and HbSS genotypes) and blood group O are reported to protect against malaria (16-18). The mechanisms by which sickle cell traits, protect against malaria are still not clear, though it is reported to interfere with the transport of parasite antigen (PfEMP1) outside the red blood cell (RBCs) (14,15). This mitigates parasite sequestration on tissues, and hence reduces disease severity (19).

The protective role of human blood grouping against malaria infections have also been reported by many studies (20-22). Some reported group O to resist against malaria infections due to reduced resetting (23,24). But current reports on ABO blood group's ability to protect against placental *P. falciparum* infections are conflicting. Therefore, further understanding of the role of ABO blood group in the pathogenesis and outcome of placental malaria is essential. Therefore, we investigated the association between HbAS and ABO blood group and placental malaria amongst pregnant women of all parities.

Materials and methods

Study site. The study was conducted in the Hohoe Municipal Hospital, in the Hohoe Municipality in the Volta Region

of Ghana. The population of this Municipality is 167,016, according to the 2010 Population and Housing Census. Malaria cases are reported throughout the year in the municipality, but the peak of cases occur in the wet seasons (April to July and September to November) with an average prevalence of 20.3% (25).

Study design, ethics and sample collection. The study was a cross sectional study conducted between July 2017 and February 2019 where 221 pregnant women were enrolled. Peripheral blood samples (4 ml) were drawn into EDTA tubes, and used for malaria smears, ABO blood grouping, dried blood blots for malaria parasites identification using PCR and electrophoresis for Hb phenotyping. A structured questionnaire was used to capture age, gravidae and bed net usage. Baby weight extracted from labour ward records and Hb levels were measured using HaemoCue 301 machine. All pregnant women who failed to sign the informed consent forms were excluded from the study. Infant birth weight and other maternal data were extracted from the ward register.

Ethics approval and consent to participate. Ethical approval was obtained from the ethics committee of the Ghana Health Service (GHS-ERC: 06103117) and Noguchi Memorial Institute for Medical Research, University of Ghana, Accra, Ghana (NMIMR-IRB CPN 077116.17). Participation was voluntary and written informed consent was obtained from participants.

Haemoglobin electrophoresis: Normal saline (5 ml) was added to 0.5 ml RBCs and centrifuged for 3 minutes, the supernatant was discarded and the process was repeated to thoroughly wash off the plasma, leaving RBC pellets. These pellets were lysed by adding 1 ml of distilled water, which was left to stand for about 5 min. About 500 ml of electrophoresis buffer TEB (0.12 M Tris, 5 mM EDTA, 15 mM boric acid, pH 8.9) was put in the electrophoresis tank compartments. Cellulose acetate strips were placed in the electrophoresis tank, and about 5 μl of haemolysate were loaded into the corresponding designated sample areas. Controls from patients with known haemoglobinopathies were included for each run.

DNA extractions. Blood blots were made from peripheral blood samples, dried and stored in a ziplock plastic bag and kept at 4˚C. DNA was extracted with Chelex 100 per the manufacturer extraction protocol (Bio Rad, United States). Briefly, blots were size cut out from the paper into 1.5 ml microcentrifuge tubes. They were then incubated at 4˚C for 30 min in 0.05% saponin phosphate buffered Saline (PBS) and spun at 4,000 rpm for one minute. This was followed by the addition of 150 μl of 6.7% Chelex resin solution into the microcentrifuge tube containing the spot and incubated at 95˚C for 10 min. The tube was then spun for 5 min at 4,000 rpm. To a new microcentrifuge tube was added 500 μl of the final extract, labelled and stored at 4˚C for PCR analysis.

Nested PCR assay. Parasite DNA was then used in nested PCR targeting 18s ssRNA (rRNA) sequences of the Plasmodium parasites (26). In brief the first step involves adding DNA samples (10 μl) and primers (rPLU5-CTTGTTGTTGCCTTAAACTTC) and rPLU6-TTAAAATTGTTGCAGTTAAAACG) into a 20 μl PCR master mix for amplification. (It will be helpful to state the concentrations of the constituents of the master mix.). In the second step, 2 μl of the sample from the first step, was added to primer pairs targeting the different malaria species in five different PCR reactions. Agarose gel electrophoresis was used to analyse the PCR products and GelDoc Go Imaging System used for visualization.

PCR primers used for amplifying the various species DNA

Plasmodium sp.	rPLU5	CTTGTTGTTGCCTTAAACTTC
	rPLU6	TTAAAATTGTTGCAGTTAAAACG
P. falciparum	rFAL1	TTAAACTGGTTTGGGAAAACCAAATATATT
	rFAL2	ACACAATGAACTCAATCATGACTACCCGTC
P. vivax	rVIV1	CGCTTCTAGCTTAATCCACATAACTGATAC
	rVIV2	ACTTCCAAGCCGAAGCAAAGAAAGTCCTTA
P. ovale	rOVA1	ATCTCTTTTGCTATTTTTTAGTATTGGAGA
	rOVA2	GGAAAAGGACACATTAATTGTATCCTAATG
P. malariae	rMAL1	ATAACATAGTTGTACGTTAAGAATAACCGC
	rMAL2	AAAATTCCCATGCATAAAAAATTATACAAA

Statistical analysis. Data was entered into Excel spreadsheets and descriptive statistics performed for all variables. Relations of blood groups with malaria and haemoglobin phenotype, were determined using χ^2-test, and regression models. Statistical analyses were performed using R statistical program, version 3.3.3 and Graph prism, P-values <0.05 were considered statistically significant.

Results

Malaria and low birth weight. Prevalence of placental malaria was high in primiparae (23.5%) as compared to (16.2%) in multiparae mothers, though not statically significant (P=0.03), it translated into a significantly low Hb (P=0.02) in these mothers, who gave birth to smaller babies (P=0.01). Also, the bed-net usage was slightly higher among placental malaria negative mothers 55.6% as compared to 46.5% in mother with placental malaria (Table I).

Blood group o primiparous women are protected from placental malaria. There was no association between any of the blood group types and risk of PM among the pregnant women (P=0.23, OR: 1.59, 95% Cl, and 0.81-3.13). But when participants were stratified by parity, women with blood group O and who are primiparous, had an increased risk of placenta malaria (P=0.04, OR: 2.85, 95% CI, 1.12-9.01) as compared

Table I. Background information.

Variable	All women (n=221)	PM + (n=43)	PM- (n=178)	P-value
Primipara	110 (49.8)	25 (23.0)	85 (77.0)	0.03
Secondipara	46 (20.8)	10 (30.0)	36 (70.0)	
Multipara	65 (29.4)	8 (12.3)	57 (87.6)	
ITN	133 (51.1)	20 (46.5)	99 (55.6)	0.03
Hb (g/dl)	12.2	10.2	12.7	0.02
LBW (Kg)	3.2	2.7	3.3	0.01

Variables between male compare using Mann-Whitney U Test. Values in brackets represent percentage or ranges where applicable. Significant P-values are in italics (P≤0.05). PM-, placental malaria negative women; PM + placental malaria positive women. ITN, Insecticide treated bed-net; LBW, Live born weight.

Table II. Association of blood groups with placenta malaria.

Parity	PCR testing Placental malaria type	Maternal parameters				
		Blood group	Number	OR	95% Cl	P-value
All	PM+	Group O	25	1.59	0.81-3.13	0.23
		Other groups	18			
	PM-	Group O	83			
		Other groups	95			
Primiparae	PM+	Group O	20	1.95	2.12-5.01	0.04[a]
		Other groups	5			
	PM-	Group O	47			
		Other groups	38			
Multiparae	PM+	Group O	11	1.91	0.74-5.40	0.30
		Other groups	7			
	PM-	Group O	42			
		Other groups	51			

[a]Unadjusted OR; primiparous women of blood group O were compared with those with other-blood groups, PM-, placenta malaria.

with those of non-group O. However, there was no observed risk in PM in multiparous women (P=0.30, OR: 1.91, 95% CI, 0.74-5.40) (Table II).

Sickle cell not a risk of placenta malaria. The association between sickle cell trait and the risk of peripheral and placenta malaria was investigated by comparing normal haemoglobin (HbAA) and sickle cell trait (HbAS, HbSC and HbSS), using the prevalence of malaria parasitaemia quantified by PCR (Table III). Placental malaria prevalence among women with sickle cell trait (25.8%) was compared with those with HbAA (20.4%), and none of these groups were protected from PM (P=0.84, OR: 0.92, 95% Cl, 0.43-1.99), nor risk of peripheral malaria (P=0.61, OR: 0.79, 95% Cl, 0.29-1.94) between the two groups.

Women of blood group O with HbAA phenotype had higher odds of PM, compared with those of non-group O with HbAA, but this was not significant (P=0.07, OR:2.12, 95% Cl; 0.92-4.66). And women of blood group O with sickle cell trait phenotype, also had a higher odds of PM compared with those of non-group O with same trait, but again, not significant (P=1.00, OR:1.03, 95% Cl; 0.26-4.10). Primiparous women of group O and HbAA phenotype also had increased odds (P=0.62, OR: 1.32, 95% Cl; 0.47-3.6) when compared with those with other blood groups with the same phenotype, though again without significant differences. The odds of PM were similar among primiparous women of group O with sickle trait and those of other blood groups with sickle trait (P=1, OR: 0.92, 95% Cl; 0.15-5.44). Therefore, blood group in combination with heamoglobin phenotype did not increase no reduce the risk of placental malaria in the two groups (Table IV).

Discussion

This is the first time the risk of placental malaria in the presence of ABO and sickle trait is investigated in pregnant women in a malaria endemic region, Ghana. In this current study primiparous women of other-blood groups (A, B, AB), had a reduced risk of active PM infections when compared with their

Table III. Sickle cell trait and the risk of peripheral malaria.

Sample type	PCR testing Malaria parasitaemia	Maternal parameters				
		Hb genotype	Number	OR	95% Cl	P-value
Peripheral	Infection	HbAA	17			
		Sickle trait	7	0.79	0.29-1.94	0.61
	no infection	HbAA	150			
		Sickle trait	47			
Placental	Infection	HbAA	32			
		Sickle trait	11	0.92	0.43-1.99	0.84
	No infection	HbAA	135			
		Sickle trait	43			

Unadjusted OR; women with sickle cell trait compared with those with HbAA.

Table IV. Heamoglobin phenotype and Blood group as co-factors and Risk of placental malaria.

Parity	PCR testing Placental malaria type	Maternal parameters					
		Blood group	HbAA	Sickle	OR	95% Cl	P-value
All	PM+	Group O	19	7	2.12$^{\alpha}$	0.96-4.66	0.07
		Other groups	13	4	1.03$^{\beta}$	0.26-4.10	1.00
	PM-	Group O	55	27			
		Other groups	80	16			
Primiparous	PM+	Group O	13	3	1.32$^{\gamma}$	0.47-3.67	0.62
		Other groups	8	3	0.92$^{\delta}$	0.15-5.44	1.00
	PM-	Group O	32	13			
		Other groups	26	12			
Multiparous	PM+	Group O	7	3	3.43$^{\varepsilon}$	0.92-12.8	0.09
		Other groups	4	2	0.95$^{\lambda}$	0.13-7.23	1.00
	PM-	Group O	26	11			
		Other groups	5	17			

α, unadjusted OR; women with placental infection and HbAA and of blood group O, compared with those with placental infection and HbAA, but of non-blood group O. β, unadjusted OR; women with placental infection and sickle trait and of blood group O compared with those with placental infection and sickle trait, but of non-blood group O. γ, unadjusted OR; primiparous women with placental infection and HbAA and of blood group O compared with primiparous women with placental infection and HbAA, but of non-blood group O. δ, unadjusted OR; Primiparous women with placental infection and sickle trait and of blood group O compared primiparous women with placental infection and sickle trait, but of non-blood group O. ε, unadjusted OR; multiiparous women with placental infection and HbAA and of blood group O compared with multiparous women with placental infection and HbAA, but of non-blood group O. λ, unadjusted OR; multiparous women with placental infection and sickle trait and of blood group O compared multiiparous women with placental infection and sickle trait, but of non-blood group O. PM-, placenta malaria.

counterparts with group O. This observation was absent in multiparous women. A similar finding was reported in Sudan, in which pregnant women of non-blood group O were at a lower risk of both active and past PM infections (27). However, in that study multiparous women were also protected. Other studies conducted in the Gambia and Malawi reported a higher risk of active PM in primiparous women, with some reporting protection in multiparous women of blood group O (21,28). It is well established that, non-pregnant individuals of group O are protected from severe malaria disease (29-31) due to the fact that infected RBCs of blood group O have a reduced ability of rosetting with uninfected erythrocytes (31). This suggests that blood group O can only reduce malaria severity, but not symptomatic and asymptomatic infections, which are implicated in pregnancy associated malaria (32-34). Thus, pregnant women of blood group O may not be prone to severe malaria, but this might not translate into placental malaria protection. During rosetting, iRBCs prefers binding to blood group A1, with least preference for group O (31). And by inference, in a non-blood group O pregnant woman, rosetting will

reduce the chances of iRBCs reaching the microvasculature of the syncytiotrophoblasts, since many of these iRBCs would be clustered by uninfected RBCs and trapped in the intervillous space. This may reduce the chances of an iRBC sequestrating in the placenta, thus the relative protection observed in this group. The basis for these assumptions will however need to be further investigated. The possible reason why this observation was not seen among multiparous women in our study may be due to immunity from antibodies (IgG) accumulated from previous exposure to VAR2CSA antigens, which is absent in primiparous women. On the part of haemoglobin phenotypes, previous studies reported HbAS ability to disrupt binding of infected RBCs to microvascular endothelium (35-37). And since infected RBCs (iRBCs) bind to placenta tissue using VAR2CSA (38,39), a disruption of this binding could protect pregnant women from placental malaria. These are the basis on which this study was designed, to investigate the potential effect of disrupting iRBCs attachment to cell surfaces by sickle cell traits and its potential protective effect against PM. But our study could not find any potential protection from HbAS and other sickle cell trait against malaria among women delivery in Ghana.

Sickle cell traits might fail to protect against placental malaria due to the fact that PfEMP1 expression on HbAS iRBC surface is variable and irregular (41). Therefore, HbAS might only restrict the expression of some specific PfEMP1 variants, which might not be sufficient to prevent VAR2CSA binding. It was again observed by Fairhurst *et al* that not all haemoglobin CC cells inhibited malaria parasite replication (40), which suggests that, the degree of protection will depend on the number of these effective (those capable of inhibiting parasite replication) cells in the individual's blood. Also, the relatively lower flow conditions of the intervillous space might promote VAR2CSA binding (15). VAR2CSA is the only specific ligand with high affinity for CSA, which suggests that even in the presence of a limited expression of VAR2CSA on iRBC surface, binding to CSA is still possible (41). And finally, when blood group type and haemoglobin phenotype were considered as co-factors in a pregnant woman, no association was observed between the risk of PM and these factors. Even when pregnant women were grouped by parity, there was still no any association. The protectiveness of non-blood group O against placental malaria in primiparous women, was neutralized when haemoglobin phenotype was considered as a co-factor. This observation may in part be due to the sample size, which was: diluted: when the women were sub-grouped into participants of one blood group phenotype with or without a sickle trait.

Conclusion

These findings indicate that, non-blood O primiparae mothers are protected against placental malaria. Though, it is accepted that primiparae women are very susceptible to placenta malaria, those with non-blood O are protected.

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Authors' contributions

ATL and BG conceived and designed the study., BG supervised the work. ATL collected data and laboratory analysis of the study. HA, MAM and ARM assisted in the experiment and data analysis. ATL, MAM, ARM and HA wrote the paper. All authors read and approved the final manuscript.

Consent for publication

The authors have read and agreed to the content of this manuscript and its publication upon acceptance.

Conflict of interest statement

The authors declare that they have no conflict of interests.

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