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Research Paper

Analysis of clinical presentation, hematological factors, self-reported bed net usage, and malaria burden in sickle cell disease patients

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ABSTRACT

Background: Sickle cell anemia (SCA) is a severe monogenic disorder, caused by single nucleotide mutations in the hemoglobin (Hb) gene, that is prevalent in malaria endemic regions of the world. Sickle cell trait (SCT) individuals carry only one of the mutated alleles and were shown to be protected against malaria. However, defining the relative contribution of hematological, clinical, and environmental factors to the overall burden of malaria in individuals with hemoglobinopathies such as SCA has been challenging.

Methods: We hypothesized that hematological differences, clinical presentations, and self-reported bed net usage among *Plasmodium*-infected and uninfected individuals may govern overall malaria burden in individuals with sickle cell disease (SCD). We conducted a cross-sectional study in Ghana from 2014 to 2019 and described clinical presentations, hematological characteristics, and bed net use based on a comprehensive questionnaire. Hematological characteristics were compared using a parametric or nonparametric ANOVA, pending if data passed D'Agostino & Pearson normality test. When comparing only two Hb genotypes hematological characteristics a Mann−Whitney *U*-test were used. Logistic regressions and Chi-squared tests were used to compare questionnaire responses between Hb genotypes. All statistical significance was set at $p < 0.05$.

Findings: Multiple hematological parameters were significantly ($p < 0.05$) altered depending on sickle cell genotype and/or malaria status. When compared to other Hb genotypes, SCA individuals with or without malaria had significantly ($p < 0.05$) higher WBC and platelets counts and lower Hb levels. While the sickle cell genotype may affect malaria severity, SCT and SCA participants were found to significantly ($p < 0.007$) use bet nets more than HbAA participants.

Interpretations: Our findings can be utilized to enhance national guidelines for reducing the incidence of malaria especially among individuals with SCD, SCT protection and health disparities among hemoglobinopathies.

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1. Introduction

Sickle cell disease (SCD) is an inherited hemoglobinopathy disorder prevalent in malaria-endemic regions where approximately 5% of the world's population carries genes associated with hemoglobin (Hb) disorders, mainly in sub-Saharan Africa [1−3]. SCD is caused by a mutation in the beta-chain in both Hb alleles, and in hypoxic conditions, the morphology of red blood cells (RBCs) become altered [2]. Multiple clinical complications are associated with SCD, including stroke and infections [2]. Sickle cell anemia (SCA) is manifested when both Hb allele mutations S (HbS) or C (HbC) are inherited (HbSS or HbSC) [4]. Sickle cell trait (SCT) occurs when only one allele variant is inherited (HbAS or HbAC), and they often suffer no clinical complications [5]. Individuals who inherit both HbC variants (HbCC) have mild/minimal symptoms [6,7]. HbC and HbS variants are

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Research in Context

Evidence before this study

Other studies have looked at HbC or HbS mutations and their hematological parameters and clinical parameters. Often these studies have looked at some of these groups but not all the genotypes (HbAA, HbAC, HbAS, HbSC, HbSS, HbCC) addressed in this study. Furthermore, this study introduces malaria infection in addition to hemoglobin genotype status. Since there are no studies looking at all these genotypes with and without malaria there is a gap in the published work regarding these groups.

Added value of this study

To our knowledge, we are the first to report differences in hematological and clinical parameters between HbAA, HbAS, HbAC, HbSC, HbSS, and HbCC with and without malaria in one publication. Our population has 11 different groups including HbCC who is not as common. Our findings are consistent with previous publications evaluating some of the groups identified in this study. We also determined sickle cell trait and sickle cell disease individuals identified to use bed nets more often than HbAA controls. This study can be utilized to enhance national guidelines for reducing the incidence of malaria especially among individuals with sickle cell disease (SCD) and other hemoglobinopathies.

Implications of all the available evidence

Knowing the extent to which these hematological factors differ between groups may enable physicians and healthcare providers to provide appropriate treatment to SCD patients. We also determined that the SCA group is more likely to have previous malaria cases than HbAA and SCT. Here again, physicians could consider these factors when discussing how patients could protect themselves against severe malaria. Finally, we have determined that SCT and SCA individuals are more likely to use a bed net. This illustrates the need for further education regardless of sickle cell status. Our findings suggest ways to enhance national guidelines for anti-malarial measures, particularly among people with sickle cell disease, and to reduce the health disparities among people with hemoglobinopathies.

predominant in West Africa [7,8]. SCT are associated with a lower malaria mortality rate than HbAA and are considered malaria-protected [2,9].

In 2018, there were 228 million cases of malaria and approximately 405,000 deaths associated with malaria globally [10]. Africa is disproportionately affected by malaria (93%), and Ghana accounts for 4% of the global malaria burden [10,11]. In Ghana, 30% of people have SCT, and approximately 1·9% of births per year have SCD [12,13]. The hematological differences among individuals with various hemoglobinopathies mediating the severity of malaria have not been fully investigated. It is also unclear how these differences impact the infection rates of malaria in individuals with such hemoglobinopathies. We hypothesized that hematological differences, clinical presentations, and self-reported bed net use of *Plasmodium*-infected and uninfected individuals may govern overall malaria burden in SCD. To test this hypothesis, we conducted a cross-sectional study using convenience sampling to assess the hematological characteristics, previous malaria infection, bed net usage, and other clinical parameters of individuals with different Hb genotypes and compared with HbAA controls in Ghana from 2014 to 2019. We then determined the

associations between these hematological factors and malaria burden in individuals with SCD.

2. Methods

2.1. Study population

We enrolled individual-level data from the Greater Accra region in Ghana, West Africa, from Korle-Bu Teaching Hospital, and district hospitals and polyclinics: Princess Marie Louise Children's Hospital, Mamprobi Polyclinic, Ussher Fort Polyclinic, Korle-Bu Polyclinic, and LA General Hospital for the pilot study. District hospitals and polyclinics are not part of the Korle-Bu teaching hospital. The Korle-Bu Teaching Hospital is part of the University of Ghana. Samples were collected between February and November 2014 and from June 2017 to July 2019 as part of an ongoing collaborative NIH-funded SCD/malaria study between Morehouse School of Medicine (MSM), Atlanta GA, USA, and the University of Ghana (UoG).

Ethical approval was obtained from the ethics review boards of MSM, UoG College of Health Sciences, and Noguchi Memorial Institute for Medical Research. Participants were selected by convenience sampling rather than randomly. Since we used convenience sampling, this could lead to the potential bias of results. All participants above 18 years signed an informed consent form, and participants <18 years of age, guardians provided written consent [14]. HIV positive individuals, those with high Fetal Hb, pregnant women, or participants with inadequate complete blood count (CBC) information were excluded. Blood samples were obtained from individuals of both sexes and all ages with and without malaria. Each participant was given a numerical code to anonymize the data and was asked to fill a comprehensive questionnaire relative to actual malaria/SCD symptoms, bed net usage, transfusions, and clinical history that has been validated in a previous study [14].

2.2. Blood sample analysis

Blood samples were collected using 8 mL BD Vacutainer CPT tubes (BD Bioscience). Blood was separated into mononuclear white blood cells (WBCs), RBCs, and plasma, following the manufacturer's instructions. Cellulose acetate membrane electrophoresis was used to determine patients' sickle cell status [14]. The same medical questionnaire was used at all enrollment facilities. Sample CBC was determined on total blood using an *ABX Micros ES 60* 18-parameter hematology analyzer at the Department of Haematology of the Korle Bu Teaching Hospital. Malaria status was determined using Rapid Diagnostic Tests (RDTs) kits (First Response® Malaria Ag. pLDH/HRP2 Combo Card Test, WHO reference number: PQDx 0285-010-00) that detected both *Plasmodium falciparum* specific protein HRP2 and Pan (Pan LDH) which detects multiple malaria species. Blood samples parasite infection were further confirmed using thick smear microscopy. HIV status was determined with RDTs (First Response® HIV-1−2 kits). We refer to malaria infected individuals with (+) and non-infected individuals with (-) after the Hb genotypes.

2.3. Statistical analysis

GraphPad PRISM 7·04 were used for all statistical analyses unless otherwise stated. Missing data was excluded from analysis. Hb genotypes were stratified to compare hematologic profiles (outcome variable) with and without malaria. Continuous variables were summarized by means and standard deviations. A sample size analysis using WBC count data determined with a 95% confidence interval (2-sided) and Power of 80% that a minimum of 6 individuals were needed in each group using HbAA- vs HbSS- groups. Hematological characteristics (WBCs, RBCs, Hb, PLT) were compared using ANOVAs and Tukey's multiple comparison test unless characteristics did not

pass D'Agostino & Pearson normality test at which point a nonparametric ANOVA and Dunn's multiple comparison test was used. Also, a Mann−Whitney U-test was used when data was nonparametric, comparing Hb genotypes with and without malaria for hematological characteristics (Fig. S1). Chi-square or Fisher's exact test was used to determine the association for numerous categorical variables (i.e. medication, anemia, malaria status). SAS 9·4 was used to do a dichotomous logistic regression model estimated the adjusted odds ratio for the likelihood of any previous malaria episodes or use a bed net as a function of genotypes. Model 1 represents the outcome variable previous episodes of malaria (never, 1−2 times, >3 times) stratified by genotype (HbAA, SCT (HbAS and HbAC combined), and SCA (HbSS and HbSC combined)) with HbAA as the reference group and includes sex and age. In model 2, the outcome variable is previous episodes of malaria (never, 1−2 times, >3 times) and is stratified by genotypes (HbAA, SCT, and SCA) and has SCT as the reference group including sex and age. In model 3, the outcome variable is bed net usage (yes/no) and was stratified by genotypes (HbAA, SCT, and SCA) with the reference being HbAA and bed net usage also including sex and age. The models were adjusted for age or sex distributional differences for the Hb genotype groups and were corrected for multiple comparisons using the Dunnett test so that age and sex did not alter results of the model. While a Polytomous logistic regression that satisfied the proportional odds property was used to model the likelihood of having 0, 1−2, or > 3 previous malaria episodes to determine if the number of malaria episodes was altered between Hb genotypes. For analysis, participants' lack of responses to questionnaires did not show a pattern, suggesting missing data were at random and not significantly correlated to the analysis covariates. A significance value of <0·05 was used. Our manuscript adheres to STROBE reporting requirements.

3. Role of funding sources

4. Results

4.1. Demographic findings

A total of 923 independent blood samples (one sample per participant) were collected. The cohort consisted of 502 (54%) women and 421 (46%) men. Median age was 28 years (mean 29·9 years, range 6 months-95 years) (Table 1). A total of 282 (31%) had malaria. A total of 66 (7·2%) participants had severe anemia (Hb<7 g/dl), 224 (24·2%) had anemia (Hb=7−9·9 g/dl), 16 (1·7%) had severe thrombocytopenia (Platelets<50 × $10^3/\mu l$), and 144 (15·5%) had mild thrombocytopenia (Platelets= 50−150 × $10^3/\mu l$) (Table S1).

4.2. Participants with SCD genotypes, have high WBC counts, low RBC counts, and low Hb concentrations

We first compared the differences between groups of Hb genotypes for WBC, lymphocytes, monocytes, granulocytes, RBCs, Hb, hematocrit (HCT), and platelets (PLT) (Table S2). We focused on differences in WBC, RBC, PLTs counts and Hb levels between HbSS and

Table 1
Population characteristics of all study participants. Mean and standard deviation for each characteristic. Normal range for WBC is 4·5–11 × $10^3/mm^3$, granulocytes is 2·5–7 × $10^3/\mu l$, lymphocytes is 0·8–5 × $10^3/\mu l$, and monocytes is 0·08–0·95 × $10^3/\mu l$. Normal RBC range for women are 4·1–5·1 × $10^6/\mu l$ and men are 4·5–5·9 × $10^6/\mu l$. Normal range for Hb is 13·5–17·5 g/dl for men and 12·0–15·5 g/dl for woman. Finally, normal range for hematocrit is 37–52% and for PLT is 150–450 × $10^3/\mu l$.

Mean±SD	HbAA- n=274	HbAA+ n=200	HbAS- n=88	HbAS+ n=23	HbSS- n=171	HbSS+ n=33	HbAC- n=33	HbAC+ n=17	HbSC- n=61	HbSC+ n=9	HbCC- n=14
Sex (males)	193	78	52	7	91	15	25	8	21	4	8
Age (years)	32·6±11·7	27·5±20·1	35·1±11·3	23·4±20·1	25·8±8·8	24·9±10·4	31·9±9·4	23·5±16·9	32·5±14·2	43·1±18·6	35·6±9·4
WBC (x10³/mm³)	5·6±1·9	6·2±2·7	5·6±1·6	6·2±2·2	12·2±5·3	16·2±6·4	5·8±1·7	6·7±2·8	8·4±3·2	11·3±7·1	7·6±1·8
Granulocytes (x10³/μl)	2·9±1·5	4·1±2·2	3±1·2	3·8±1·8	7·1±4·2	9·5±4·8	3·1±1·1	4·5±2·9	5·1±2·6	6·8±3·3	4·1±1·6
Lymphocytes (x10³/μl)	2·1±1	1·6±1·1	2±0·7	1·8±0·8	4·2±2	5·2±2·8	2·1±0·7	1·7±0·9	2·6±1·1	3·3±3·6	2·5±0·5
Monocytes (x10³/μl)	0·6±0·8	0·6±0·4	0·6±0·7	0·7±0·4	0·9±0·9	1·5±0·9	0·7±0·3	0·6±0·2	0·6±0·4	1·2±0·9	1±0·3
RBC (x10⁶/μl)	5±0·9	4·2±0·7	5±0·8	4·4±0·7	3·1±0·8	2·7±0·9	5±1	4·6±0·9	4·2±0·9	3·7±0·7	4·7±0·6
Hb (g/dl)	14±2·5	11·4±2·2	13·7±2·8	11·6±1·9	8·1±1·5	7·3±2·1	14·3±2·4	11·3±2·2	10·5±1·7	9·5±1·9	12·1±1·5
Hematocrit (%)	39·7±5·7	35·1±7·9	39·8±5·1	35·5±6·6	25·7±4·9	22·6±5·8	38·2±6·9	34·8±8	34·3±5·9	32·2±8	38·9±4·5
PLT (x10³/μl)	296·7±108·6	159·1±91·7	280·1±93·8	180·7±99·2	433±177·1	383·6±142·9	290·8±90·6	154·7±67·5	295±117·2	220·4±136·6	207·1±57·1

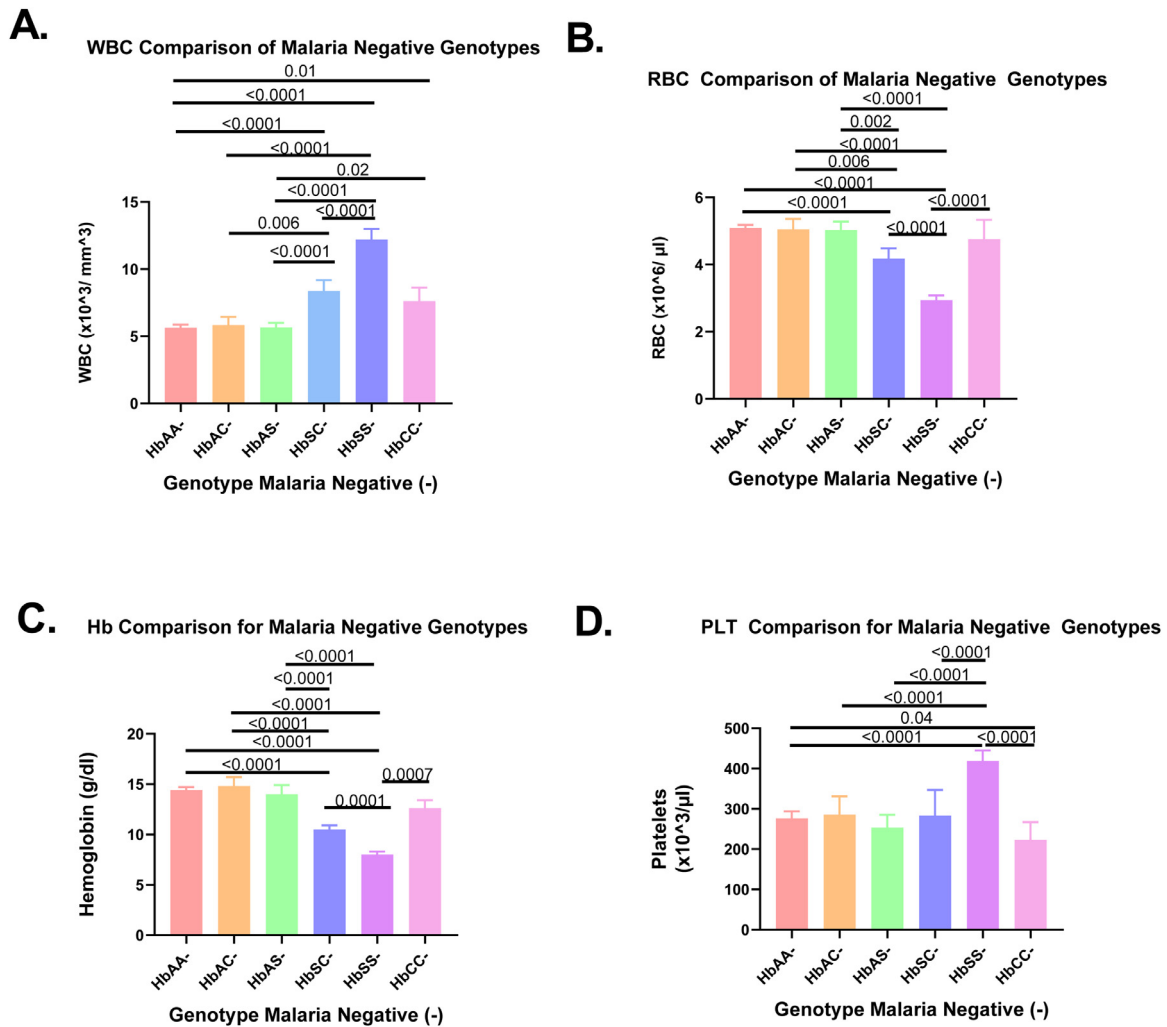HbAA+ column: N = 199. HbAC+ column: N = 16.

Fig. 1. WBC, RBC, PLT counts and Hb levels in uninfected participants are dependent on Hb genotypes. The data did not pass D'Agostino & Pearson normality test thus a nonparametric ANOVA and Dunns' comparison test was used to determine if there was a significant difference between groups. Standard deviation is illustrated by the error bars. We first assessed variation between groups of uninfected Hb genotypes for WBC, lymphocytes, monocytes, granulocytes, RBCs, Hb, hematocrit (HCT), and platelets (PLT) (Table S2). WBC, RBC, PLT counts and Hb levels were determined using *ABX Micros ES 60* 18-parameter hematology analyzer as described in the Methods section. A. WBC counts were compared among uninfected participants with HbSC- ($n = 61$), HbSS- ($n = 171$), HbAA- ($n = 274$), HbAC- ($n = 33$) and HbAS- ($n = 88$) genotypes. B. RBC counts were compared among uninfected participants with HbSC- ($n = 61$), HbSS- ($n = 171$), HbAA- ($n = 274$), HbAC- ($n = 33$) and HbAS- ($n = 88$) genotypes. C. Hb levels were compared among uninfected participants with HbSC- ($n = 61$), HbSS- ($n = 171$), HbAA- ($n = 274$), HbAC- ($n = 33$) and HbAS- ($n = 88$) genotypes. D. PLT counts were compared among uninfected participants with HbSC- ($n = 61$), HbSS- ($n = 171$), HbAA- ($n = 274$), HbAC- ($n = 33$) and HbAS- ($n = 88$) genotypes.

HbSC participants (Table S2). WBC counts were highest in HbSS- [12.2 ± 5.3] followed by HbSC- [8.4 ± 3.2] and HbCC- [7.6 ± 1.8] (Fig. 1A). There was no difference in WBC counts among the HbAA- [5.6 ± 1.9], HbAC- [5.8 ± 1.7] and HbAS- [5.6 ± 1.6] genotypes which had counts in the normal range ($4.5-11 \times 10^3/\text{mm}^3$). RBC counts were lowest in HbSS- [3.1 ± 0.8] and HbSC- [4.2 ± 0.9] participants when compared to all other non-malaria Hb genotypes groups which were at normal range ($4.1-5.9 \times 10^6/\mu L$) (Fig. 1B). Hb levels were significantly lower ($p < 0.005$) in HbSC- [10.5 ± 1.7] and HbSS- [8.1 ± 1.5] when compared with levels in HbAA- [14 ± 2.5], HbAC- [14.3 ± 2.4], HbAS- [13.7 ± 2.8] and HbCC- [12.1 ± 1.5] which were at normal range ($12-15.5$ g/dl) (Fig. 1C). PLT counts was significantly higher ($p < 0.05$) in HbSS- [433 ± 177.1] compared with counts in HbAC- [290.8 ± 90.6], HbAA- [$296.7.1 \pm 108.6$], HbAS- [280.1 ± 93.8], HbCC- [207.1 ± 57.1] and HbSC- [295 ± 117.2] which were in normal range ($150-450 \times 10^3/\mu L$) (Fig. 1D). Interestingly, PLT count in HbCC- [207.1 ± 57.1] genotype was the lowest among all Hb genotypes screened. Finally, hematocrit was lowest in HbSS- [$25.7\% \pm 4.9$] compared to HbAA- [$39.7\% \pm 5.7$], HbAS- [$39.8\% \pm 5.1$],

HbAC- [$38.2\% \pm 6.9$], HbSC- [$34.8\% \pm 5.9$] and HbCC- [$38.9\% \pm 4.5$] genotypes that were at normal range [$36-50\%$]. (Table S2).

4.3. Plasmodium falciparum infected study participants with SCD genotypes have lower levels of Hb and RBC counts but higher WBC counts compared to HbAA controls

During the study period, we did not encounter participants with HbCC and malaria (HbCC+). WBC counts were significantly higher ($p < 0.0001$) in HbSS+ [16.2 ± 6.4] compared to HbAA+ [6.2 ± 2.7]. WBC counts in HbAC+ [6.7 ± 2.8] and HbAS+ [6.2 ± 2.2] genotypes were also above those in uninfected participants (Fig. 2A). RBC counts were generally low in infected participants (Fig. 2B). However, counts were lowest in HbSS+ [2.7 ± 0.9] followed by HbSC+ [3.7 ± 0.7] participants when compared to HbAA+ [4.2 ± 0.7], HbAC+ [4.6 ± 0.9], and HbAS+ [4.4 ± 0.7] genotypes (Fig. 2B). Hb levels were significantly lower ($p < 0.0001$) in HbSS+ [7.3 ± 2.1] when compared with levels in HbAA+ [11.4 ± 2.2], HbAC+ [11.3 ± 2.2], and HbAS+ [11.6 ± 1.9] genotypes (Fig. 2C). Platelet count was highest in HbSS+

A.

WBC Comparison of Malaria Positive Genotypes

B.

RBC Comparison for Malaria Positive Genotype

C.

Hb Comparison for Malaria Positive Genotypes

D.

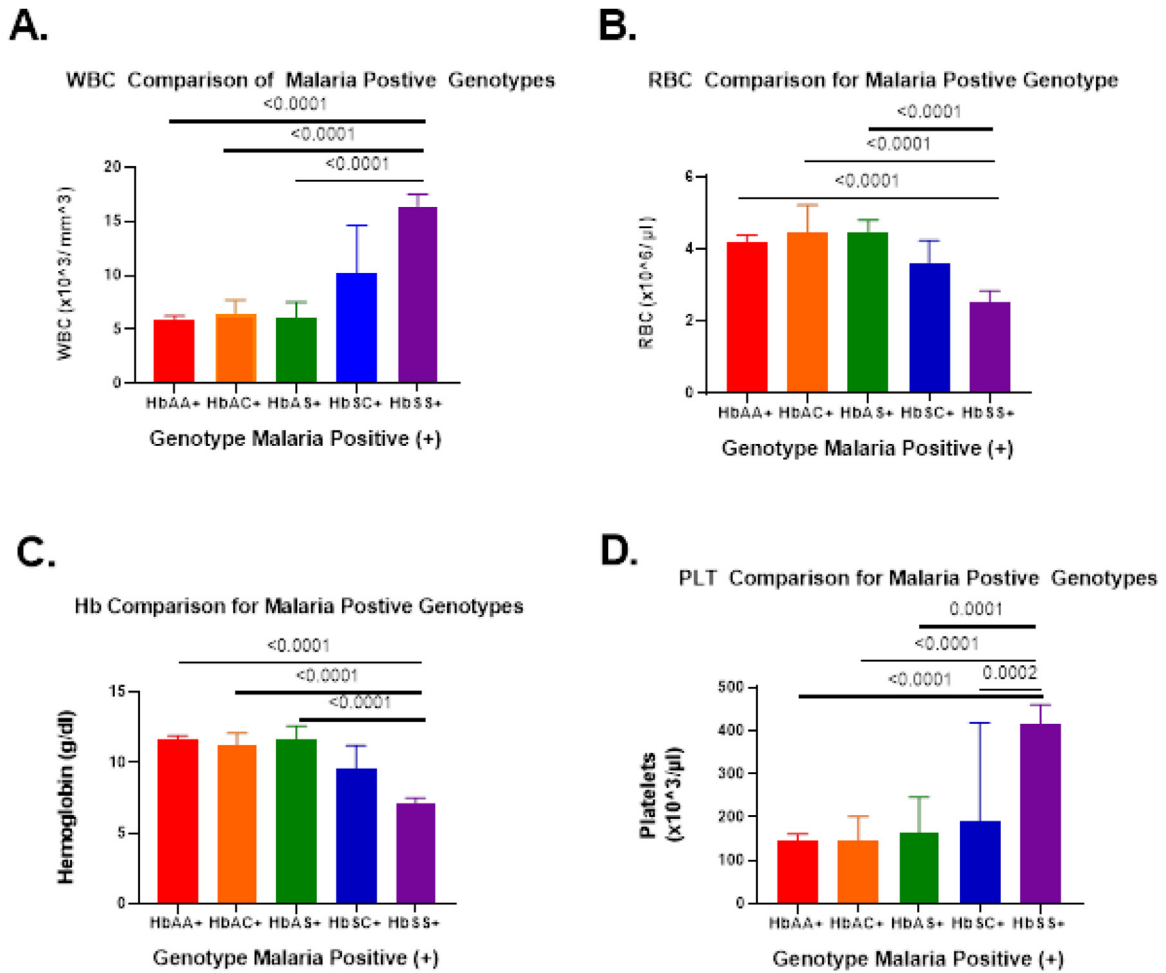### PLT Comparison for Malaria Positive Genotypes

Fig. 2. WBC, RBC, PLT counts and Hb levels are altered among *P. falciparum* infected genotypes. The data did not pass D'Agostino & Pearson normality test thus a nonparametric ANOVA and Dunns' test was conducted to analyze variation between groups. For PLT, an ordinary one-way ANOVA and Tukey's multiple comparison test was used. Standard deviation is illustrated by the error bars. We first compared the differences between groups of malaria infected Hb genotypes for WBC, lymphocytes, monocytes, granulocytes, RBCs, Hb, hematocrit (HCT), and platelets (PLT) (Table S3). WBC, RBC, PLT counts and Hb levels were determined using *ABX Micros ES 60* 18-parameter hematology analyzer as described in the Methods section. A. WBC counts were determined among malaria infected participants with HbSC+ ($n = 9$), HbSS+ ($n = 33$), HbAA+ ($n = 200$), HbAC+ ($n = 17$) and HbAS+ ($n = 23$) genotypes. B. RBC counts were determined among malaria infected participants with HbSC+ ($n = 9$), HbSS+ ($n = 33$), HbAA+ ($n = 200$), HbAC+ ($n = 17$) and HbAS+ ($n = 23$) genotypes. C. Hb levels were determined among malaria infected participants with HbSC+ ($n = 9$), HbSS+ ($n = 33$), HbAA+ ($n = 200$), HbAC+ ($n = 17$) and HbAS+ ($n = 23$) genotypes. D. PLT counts were determined among malaria infected participants with HbSC+ ($n = 9$), HbSS+ ($n = 33$), HbAA+ ($n = 199$), HbAC+ ($n = 17$) and HbAS+ ($n = 23$) genotypes.

[383·6 ± 142·9] followed by HbSC+ [220·4 ± 136·6] compared with counts in HbAC+ [154·7 ± 67·5], HbAA+ [159·1 ± 91·7], and HbAS+ [180·7 ± 99·2] (Fig. 2D). Hematocrit was lowest in HbSS+ [22·6% ± 5·8] compared to HbAA+ [35·1% ± 7·9], HbAS+ [35·5% ± 6·6], HbAC+ [34·8% ± 8], and HbSC+ [32·62% ± 8] genotypes [36−50%] (Table S3).

4.4. Overall trends for Hb levels, WBC, RBC, and PLT counts remain steady within Hb genotypes but are altered by P. falciparum infection

The overall trends described above for WBC, RBC, and PLT counts as well as for Hb levels remained the same for all genotypes with and without malaria (Table S2-4,Fig. S1). Of note granulocyte counts were elevated ($p < 0.0001$) in HbAA+ [4·1 ± 2·2] compared to HbAA- [2·9 ± 1·5] and concordant with decreased lymphocytes, RBC, Hb, HCT, and PLTs. Besides PLT, all CBC variables showed significant alteration ($p < 0.0001$) when HbAA- was compared to HbSS+. When HbAA- and HbSC+ were compared, WBC and granulocytes were significantly elevated, whereas RBC, Hb, and HCT (Table S4) were decreased. Also, granulocytes were higher in HbAC- than HbSC+, whereas RBC and Hb (Table S4) were lowered.

All CBC variables were significantly increased or decreased depending on the hematological status HbSS- vs HbAA+($p < 0.0001$). WBC, lymphocytes, granulocytes, and PLT were significantly elevated

($p < 0.05$), with a corresponding decrease in Hb levels when HbSC- was compared with HbAA+. The CBC variables remained unchanged when HbSC- was compared to HbSC+ (Table S4). HbSS- and HbSC- genotypes had the most alterations in hematological parameters when compared to other genotypes.

We also assessed whether WBC, RBC, PLT counts and Hb levels were altered in relation to Hb genotype when individuals become infected with *P. falciparum*. We compared WBC, RBC, PLT, and Hb values for each genotype independently, with and without *P. falciparum* infection (Fig. S1). WBC counts were significantly increased ($p < 0.0001$) when uninfected HbSS- [12·2 ± 5·3] individuals became infected (HbSS+) [16·2 ± 6·4] (Fig. S1Q). RBC counts and Hb levels on the other hand significantly declined ($p = 0.0007$) accordingly (Fig. S1R-S).

4.5. Individuals with SCA report higher incidence of malaria than individuals with SCT or HbAA

In our survey, 297 out of 923 responded to the question relating to the frequency of symptomatic malaria experienced in their lifetime. Two hundred ninety-two reported using a bed net or not (Table 2). Of the participants assessed for previous malaria cases, 58% were female, 37% HbAA, 10·4% SCT, 51·9% SCA, 0·7% HbCC, and 41% were

Table 2
Assessment of previous malaria episodes and bed net usage. Data is categorized by age, sex, genotype, and malaria status at time of visit.

	Previous cases of malaria					Mosquito Net		
	All N = 297	Never N = 38	1−2 Times N = 90	>3 Times N = 117	Yes N = 259	All N = 292	Yes N = 59	No N = 233
Age (Mean±SD)	29·1 ± 14·6	27·2 ± 21·3	27·8 ± 12·8	28·9 ± 12·5	29·3 ± 13·4	29·5 ± 14·6	29·9 ± 15·2	27·9 ± 12·3
Sex (female)	173	22	51	62	151	166	36	130
	58%	58%	57%	53%	58%	57%	61%	56%
Genotype								
HbAA	110	24	34	23	86	88	7	81
	37%	63%	37%	20%	33·2%	30·2%	12%	35%
SCT (HbAS &HbAC)	31	6	11	10	25	38	14	24
	10·4%	16%	12%	9%	10%	13%	24%	10%
SCA (HbSS & HbSC)	154	8	45	84	146	156	37	119
	51·9%	21%	50%	71%	56%	53·4%	63%	51%
HbCC	2	0	1	0	2	10	1	9
	0·7%	0%	1%	0%	0·8%	3·4%	1%	4%
Malaria positive at time of visit	122	26	34	29	96	105	15	90
	41%	38%	38%	25%	37%	36%	25%	39%

malaria positive at the time of visit. Among those assessed for bed net use, 57% were female, 30·2% HbAA, 13% SCT, 53·4% SCA, and 3·4% HbCC. Thirty six percent were malaria positive at the time of the visit (Table 2).

Model 1 represent previous episodes of malaria stratified by genotype with HbAA as the reference group. The odds ratio of the SCA group having previous episodes ($\geq$1 episode) of malaria is 4·6 times (95% CI 2·66 − 8) as likely as HbAA (Table 3, Fig. 3A). The likelihood of a SCD individual having > 3 malaria episodes is 0·86 ($p = 0.0001$), and for 1−2 malaria episodes are 0·98 ($p < 0.0001$) (Table 4). The likelihood of an HbAA having > 3 times malaria episodes is 0·22 ($p = 0.0006$) and for 1−2 times is 0·68 ($p = 0.03$). The likelihood of a SCT individual having 1−2 times malaria episodes is 0·9 ($p < 0.0001$) (Table 4). Interestingly, SCT individuals reported a lower frequency of >3 malaria episodes then HbAA. This may illustrate the protection seen in SCT individuals against malaria. While model 2 represent previous episodes of malaria stratified by genotype with SCT (HbAS and HbAC) as the reference group. In model 2, the odds ratio of the SCA groups having episodes ($\geq$1 episode) of malaria is 2·9 times (95% CI 1·3−6·4) as likely as SCT (Table 3, Fig. 3B). Overall, the model illustrates that SCD individuals reported a higher frequency of multiple malaria episodes compared to HbAA.

4.6. Individuals with SCT report a higher frequency of bed net use than SCA or HbAA individuals

Model 3 represent bed net usage stratified by genotype with HbAA as the reference group. The odds that the SCA group will use a bed net are 3·7 times greater than that for the HbAA group (Table 3, Fig. 3C). The odds of a bed net use in SCT were 7·24 times higher than in HbAA (Table 3, Fig. 3C). When HbCC was accounted for in the bed net usage model, there was no significant difference ($p = 0.69$). Additionally, we compared genotypes to previous malaria cases by controlling for bed net usage. We found that bed net usage had no significant association with previous malaria cases ($p = 0.46$). Therefore, this model suggests that SCT individuals use bed nets more frequently than SCA or HbAA individuals.

4.7. HbSS cases are more severe than HbSC

We utilized the need for transfusions and medications, RBC counts <4·5 for men and <4·1 for women, and Hb levels <13 g/dl for men and <12 g/dl for women as markers of severity. We examined the relation between SCA genotypes (HbSS and HbSC) and blood transfusion history ($X^2 = 26.4$, $p < 0.0001$) (Table S5) and determined that HbSS (48%) individuals had more blood transfusions then HbSC (6%) individuals. We also examined the relationship between SCA genotypes (HbSS and HbSC) and SCD complications in those receiving a transfusion ($X^2 = 20$, $p < 0.0001$) but negative for malaria test at the time of their visit. HbSS (43%) individuals had more transfusions than HbSC (6%) individuals when stratified for individuals reporting suffering SCD complications (Table S6). Anemia status at the time of the visit correlated with SCA genotype and SCD complications with a negative malaria diagnosis was $X^2 = 59.8$, $p < 0.0001$. This suggests that HbSS (67%) individuals were more likely to be anemic (RBC<4·5 $\times$ $10^6/\mu L$ for men and <4·1 $\times$ $10^6/\mu L$ for women), compared to HbSC (13%) (Table S6). However, there was no significant difference in anemia determined by Hb levels (<12 g/dL in women and <13·5 g/dL in men) among HbSC and HbSS (Table S6). This confirms previous reports of HbSS being more severe then HbSC [15].

Patients were categorized by Hb level and PLT counts [16] (Table S1). Most individuals, regardless of genotype or malaria status, had Hb level $\geq$10 and PLT count >150. Of note, HbSS- and HbSS+ had Hb levels <7 although HbAA+, HbAS+, and HbAC+ had severe thrombocytopenia (<50).

The prevalence of self-reported SCD complications was the same in HbSC and HbSS patients regardless of malaria status (Table S7). As a result, individuals with malaria had different malaria symptoms
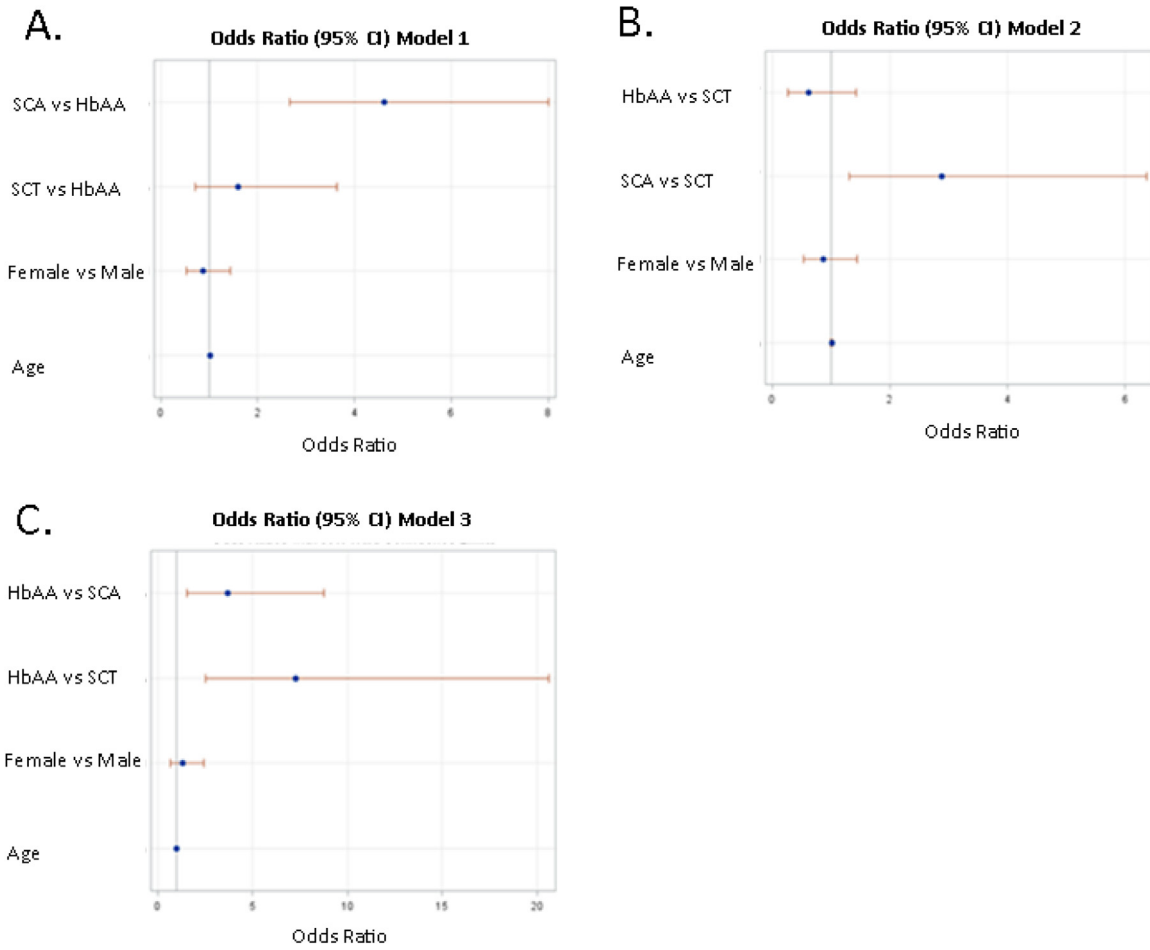
Table 3
Adjusted odds ratio for three models used to analyze previous episodes of malaria and bet net usage among different genotypes. All models were adjusted for differences in age and sex among genotypes. All models were adjusted for multiple comparisons using Dunnett test. The proportional odds assumption was satisfied. SCT includes genotypes HbAS and HbAC. SCA includes genotypes HbSC and HbSS. Model 1 is the adjusted odds ratio for previous positive malaria diagnosis with HbAA as the reference group. Model 2 is the adjusted odds ratio for previous malaria diagnosis with SCT as the reference group. Model 3 is the adjusted odds ratio for bed net usage but excludes genotype HbCC.

Adjusted Odds Ratio	OR	95% CI	Adjusted P value
Model 1: Previous malaria episodes (ref=HbAA)			
SCA vs HbAA	4·6	2·66−8	<0·0001
SCT vs HbAA	1·6	0·7−3·6	0·26
F vs M	0·87	0·53−1·4	0·58
Age	1	0·99−1·03	0·26
Model 2: Previous malaria episodes (ref=SCT)			
HbAA vs SCT	0·65	0·3−1·4	0·26
SCA vs SCT	2·9	1·3−6·4	0·02
F vs M	0·87	0·5−1·4	0·59
Age	1	0·9−1·03	0·2
Model 3: Mosquito net usage (excludes HbCC) (ref=yes & HbAA)			
SCT vs HbAA	7·2	2·5−20·6	0·0004
SCA vs HbAA	3·7	1·6−8·8	0·006
F vs M	1·3	0·7−2·4	0·43
Age	0·98	0·9−1	0·14

Fig. 3. Adjusted odds ratio and 95% CI for previous cases of malaria and bed net usage. All models were adjusted for distributional differences in age and sex among genotypes. The proportional odds assumption was satisfied. SCT includes genotypes HbAS and HbAC. SCA includes genotypes HbSC and HbSS. A. Model 1 is the adjusted odds ratio for previous malaria cases with HbAA as the reference group. The odds of the SCA group having previous episodes of malaria are higher than HbAA. B. Model 2 is the adjusted odds ratio for previous malaria cases with SCT as the reference group. The odds of the SCA group having previous episodes of malaria are higher than SCT. C. Model 3 is the adjusted odds ratio for bed net usage but excludes genotype HbCC. The odds of SCA and SCT using a bed net is more than HbAA.

Table 4

SCD has more previous cases of malaria then HbAA and SCT. Model 1 uses HbAA as the reference group. Model 2 uses SCT as the reference group. Pr=Probability.

	Malaria Episodes			
	> 3 Times		1−2 Times	
	Pr	P value	Pr	P value
Model 1: Previous malaria episodes (ref=HbAA)				
HbAA	0.22	0.0006	0.68	0.03
SCT	0.57	0.35	0.9	<0.0001
SCD	0.86	0.0001	0.98	<0.0001
Model 2: Previous malaria episodes (ref=SCT)				
HbAA	0.32	0.08	0.77	0.007
SCT	0.22	0.0006	0.67	0.04
SCD	0.15	0.008	0.57	0.66

based on their genotype (Table S8). Specifically, SCA+ (HbSS+ and HbSC+) reported less seizure/convulsion (X^2 = 17·4, p = 0·0002) and red urine (X^2 = 20·9, $p < 0.0001$) as a symptom compared to HbAA+ and SCT+ (HbAS+ and HbAC+). Also, HbAA+ self-reported red urine as a symptom of malaria more than SCT+ and SCA+ (Table S8). This is interesting since, in theory, SCA would be more severe with SCD and malaria combined as compared to HbAA+, so we would expect SCA individuals to experience more malaria symptoms.

5. Discussion

In this study, we examined the hematological differences between individuals with HbAA, HbAS, HbAC, HbSC, HbSS, and HbCC Hb genotypes that were either infected with (+) or without (-) malaria (Table 1). Our results indicate that HbSS-, HbSC-, and HbCC- individuals have increased WBC counts than HbAA- and SCT- (Fig. 1A). The reported higher WBC to reflect expected increased chronic inflammation and corresponding cytokine response due to SCA associated chronic hemolysis [15,17,18]. Interestingly, a subset of this cohort showed altered expression of inflammatory cytokines, including CXCL10, TNF-α, CCL2, IL-8, and IL-6, associated with hemoglobin genotypes and *P. falciparum* infections [18]. The increased PLT counts in HbSS- were significantly higher ($p < 0.05$) than observed for all the other genotypes, due to reduced splenic sequestration of PLT and increased erythropoietin associated with SCA (Fig. 1D) [19,20].

RBC counts for HbSC- and HbSS- were significantly ($p < 0.007$) lower than HbAA-, HbAS-, and HbAC- (Fig. 1B). HbCC- had slightly lower RBC counts compared to HbAA-, HbAS-, and HbAC-. This is consistent with the carriage of two HbC alleles (HbCC), which are associated with mild symptoms (Fig. 1B) [6,7]. Since the HbCC genotype predisposes individuals to mild symptoms, they have similar RBC counts observed in HbAA, HbAC, HbAS, or HbSC [6,7,21]. RBCs in SCA individuals have a shorter life span than HbAA due to hemolysis [15,17,22]. Less RBC results in lower Hb levels [3]. Therefore, the trends seen whereby RBC counts and Hb levels are decreased in HbSC

and HbSS are consistent with SCA pathology and shorter life span of RBC [23] as previously reported [15–17].

The reduction of RBC counts and Hb levels in malaria patients is not surprising due to the RBC damage associated with parasite replication, which occurs faster in individuals with hemoglobinopathies [10]. Thus, RBC counts are significantly decreased for hemoglobin genotypes HbAA, HbAS, and HbSS infected with malaria (Table S3-4) [10,16]. We observed decreasing RBC counts for all Hb genotypes infected with *P. falciparum*. SCT RBC's have reduced polymerization since only half their Hb is mutated [7]. SCT individuals maintain RBC homeostasis via heme detoxification, limiting RBC destruction, and heme levels [24,25]. The differences between HbAC- and HbAC+ may be due to the extent of interaction between HbAC RBC's and parasites, which confers protection against malaria [26,27]. Furthermore, the lack of a difference in WBC counts between HbAC- and HbAC+ may indicate a compensatory dampening of inflammation associated with these genotypes [26,27].

HbC creates crystals in RBC, and the crystals melt under deoxygenated conditions and do not cause vasoocclusion as observed in HbS [7]. HbSC RBC's possess HbS and HbC variants, thus lowering the concentration of HbS compared to HbSS [7]. It seems that infection of HbSC RBC's may result in limited alteration of RBC when compared with HbSS. The increased WBC and decreased RBC counts and Hb levels could be due to reduced RBC lifespan and increased inflammation due to parasite in HbSS individuals [10]. Identifying these hematological differences enables a deeper understanding of malaria interplay in SCD pathogenesis and factors mediating protection against malaria.

Our questionnaire used two variables, previous malaria, and bed net usage cases, to assess malaria exposure in different Hb genotypes. SCA individuals were 4·6 times ($p < 0·0001$) likely to have self-reported previous cases of malaria than HbAA (Table 3, Fig. 3A), suggesting SCA individuals in this cohort carry a higher malaria burden. While the literature reported SCD patients had a reduced risk of contracting malaria, these studies were conducted in children, whereas our research evaluated all ages [28,29].

SCT and SCA individuals were 7·2 and 3·7 times ($p < 0·007$) more likely to use a bed net than HbAA, respectively (Tables 2 and 3, Fig. 3C and D). The increased bed net usage could be due to better education in SCD communities about increased malaria mortality in SCD [26]. Examining previous malaria cases and adjusting for bed net usage, we determined that bed net did not correlate with the frequency of self-reported prior infection. However, combining malaria episodes and bet net usage in one model is not representative of all malaria preventative measures that are used. Therefore, it is not the best way to analyze bet net usage, and self-report previous malaria cases reported. However, when used as an outcome, we purposely omitted the malaria episodes and clinical/health conditions from the bed net usage models to determine if certain Hb genotype status is more likely to report using a bed net while adjusting for sex and age. If bed net usage had been used as an outcome in a model including baseline clinical/health conditions and malaria burden, bed net use would be a confounder, and other malaria preventions would have also needed to be added to the model. Therefore, our model evaluating bed net usage among individuals with different Hb genotypes, suggests certain Hb genotypes are reporting using bed nets more than other Hb genotypes. This could suggest that education on bed net use in SCT and SCA communities could potentially account for this increase in bed net usage reported.

HbSS individuals had more transfusions because they suffer severe forms [21]. In this study, the HbSS group had more anemia than HbSC (Table S6), which is consistent with the literature [15,21,26]. This could be due to the HbC allele making them less symptomatic.

The study had some limitations. First, participants were selected by convenience sampling rather than randomly. Since we used convenience sampling, this could lead to the potential bias of results. We enrolled subjects from the SCD clinic to have access to all Hb genotypes. Since we enrolled subjects from a SCD clinic, we could have more SCD volunteers than the actual percentage in the population. Another limitation is that we mostly used self-reported symptoms, and some questionnaires were incomplete, decreasing our sample size. While there was no pattern in lack of response to the questionnaire questions, it does limit our power when making comparisons and creating models. It was not easy to obtain information from participants' records thus we only used self-reported health information collected via our surveys. Since this is a cross-sectional study and not prospective, this leads to potential information not reported in our questionnaire, unreported health complications and disease history, that could alter hematological parameters. Overall, these limitations could have led to bias in the hematological parameters at a population level. Furthermore, some comparative groups had smaller sample sizes due to the number of subgroupings, which reduced our power for certain comparisons. Nevertheless, the cohort and data presented here illustrate significant diversity in hematological and behavioral factors that translate into susceptibility to malaria that has not been previously reported.

We are the first to show that hematological differences occur in individuals with HbAA, HbAS, HbAC, HbSC, HbSS, and HbCC as part of one comparison [15,26,28]. Moreover, we determined that the SCA group is more likely to have had previous malaria cases than the HbAA and SCT groups. Based on the results of this pilot study, the hematological variations between different Hb sickle genotypes and malaria could be generalized to the Ghanaian population and be applicable to other African countries. The study offers a benefit to clinicians by controlling inflammation through hematological testing, which could be used by physicians to advise patients on how to avoid severe malaria. We have also found that people with SCT and SCA are more likely to use bed nets than people with HbAA. This illustrates the importance of further education regardless of sickle cell status on benefits of using bed nets. Our findings suggest ways to improve national anti-malarial guidelines, particularly for people living with sickle cell disease, and to reduce health disparities among people with hemoglobinopathies.

Contributors

AD, JKS, and KOH designed the study. AD, MDW, YDA, AAA, and FB organized patient recruitment, sample collection, storage, and shipment. KOH and AD conducted the experiments. KOH, MM, AD, and JKS analyzed and interpreted the data. KOH and AD wrote the paper. FB, MDW, YDA, AAA, WET, JKS, and AD edited and approved final manuscript.

Data Sharing Statement

For original deidentified data, please contact adel.driss@gmail.com. Senior author Adel Driss has verified and is responsible for the raw data and access to the data.

Declaration of Competing Interest

No competing interest.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.eclinm.2021.101045.

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