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The Influence of Hemoglobin Variants on *Plasmodium falciparum* Parasite Density

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Authors' contributions

This work was carried out in collaboration among all authors. Author LMM initiated, designed, assessed the data and wrote the first draft of the study. Authors MSY, IHA, ADMGOA, DM and MLI collected the data and revised the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Malaria and sickle cell disease are public health problem in sub-Saharan Africa. We study the influence of hemoglobin type on parasite density in suspected sickle cell malaria patients in Maradi (Niger). This was a descriptive study with retrospective data collection between 2012 and 2023. Electrophoresis methods were used to determine the hemoglobin type and thick smear for the parasite density. This study included 875 participants with a sex ratio of 1.06 and a mean age of 14.25 years (range: 2 months to 80 years). Thick smear analysis of all participants revealed 52.91% positive, and the arithmetic mean of 242 p/uL (40 p/uL - 2600 p/uL). The most prevalent hemoglobin types were hemoglobin A (66.17%), following with hemoglobin S (29.14%) and hemoglobin C (3.66%). The geometric mean of the parasite density applied to the hemoglobin type shows that hemoglobin C (289.65 p/uL) and hemoglobin S (291.39 p/uL) stand out as being the highest. These results show that the differences in parasite density between hemoglobin A, hemoglobin S and hemoglobin C are statistically significant (p= 2.28x10-59). Regression analysis showed that hemoglobin C had a significant positive influence (p=0.029) on parasite density. The hemoglobin A2, hemoglobin F and hemoglobin S, did not have a statistically significant impact on parasite density. According to this study, person with predominant hemoglobin S and hemoglobin C have highest parasitemia than patients with predominant hemoglobin A type. It's necessary to conduct others studies to determine the mechanism by how hemoglobin type affects parasite density in malaria.



Keywords: Malaria; sickle cell disease; hemoglobin C; parasite density.

1. INTRODUCTION

Malaria remains a major public health problem in Africa, with almost 234 million cases in 2021. It is a major parasitic pandemic caused by parasites of the Plasmodium genus (Organization, 2023). distribution, although random in Its the population, seems to coincide with that of sickle cell disease, a clinically and co-dominantly autosomal recessive hereditary disease that is biologically transmissible (Makani et al., 2007; Esoh & Wonkam, 2021). According to the WHO, sickle cell disease is the most widespread genetic disorder in the world. In Africa, the prevalence of the gene responsible varies from 10 to 40%, making it the most widespread

genetic disease, with 79,200,000 people affected. A thousand children are born with the disease every day, and more than half of them will die before the age of five (Piel et al., 2013). HbS is widespread in sub-Saharan Africa and Asia, while HbC occurs only in a small area of central West-Africa (Modiano et al., 2007). Although sickle cell sufferers have a lower malaria-related mortality rate than non-sickle cell sufferers. they nevertheless suffer more frequently from malaria-related complications (Daou et al., 2019; Eleonore et al., 2020). In Niger, the prevalence rate of haemoglobin S carriage is 25% (Malam-Abdou et al., 2016). Numerous studies have been and continue to be carried out to understand the relationship

between sickle cell disease and malaria. in particular the impact of different hemoglobin variants (HbS, HbC and HbE) against severe malaria (Eridani, 2013; López et al., 2010). The interaction between these different genotypes and parasite density is less well studied and should receive particular attention because of the potential of hemoglobin types to modulate parasite density, which is a frank indicator of parasite load that directly influences the severity of malaria. Gaining a deeper understanding of potentially protective the mechanisms of hemoglobin variants and their role in resistance/susceptibility and malaria transmission could contribute to the development of targeted treatments and effective public health strategies. Hemoglobin variants could protect the host against severe malaria but may also increase transmission of the pathogen to the Anopheles vector (Pasvol, 2010). The varied geographical distribution of the different hemoglobin forms highlights the need for a regional understanding in order to implement regional strategies adapted to each context (Modiano et al., 2007; Piel et al., 2013). A molecular study has shown that hemoglobin S and C interact via host microRNA and the reading of Plasmodium falciparum proteins that remodel red blood cells, transport parasite proteins to their surface and induce immunity in the host (Taylor et al., 2012). A study on miRNA has shown that the severity of sickle cell disease and malaria could be modulated by miR-451a and let-7i-5p (Oxendine Harp et al., 2023). In northern Ghana it has been shown that, when the AS and AC genotypes co-exist with Athalassaemia, this increases the risk of asymptomatic parasitemia (Lamptey et al., 2023). In Burkina Faso study showed that the prevalence of these genetic factors was low among children with high parasite density (Badoum et al., 2019).

The aim of this study was therefore to bridge the gap between *Plasmodium* density and the predominant hemoglobin type by providing concrete data on how specific hemoglobin variants, including HbA, HbA2, HbS, HbC, HbF, as well as the presence or absence of the sickle cell trait, influence parasite density.

2. MATERIALS AND METHODS

2.1 Study Site

Maradi region is located in south-central Niger, 645 km from the capital Niamey, between parallels 13° and 15°26' north latitude and 6°16' and 8°36' east longitude. It is bordered to the east by the Zinder region, to the west by the Tahoua region, to the north by the Agadez region and to the south by the Federal Republic of Nigeria, with which it shares a border of around 150 km (Fig.1).

2.2 Study Design and Population

We conduct an observational retrospective study that focused on data collection from the year 2012-2023. The study population consisted of individuals residing in Maradi region who had undergone thick smear and electrophoresis. Venous blood collected in EDTA tubes was used for analysis of the electrophoretic profile of hemoglobin and the thick smear.

2.3 Laboratory Methods

2.3.1 Performing electrophoresis

For optimum results, the red blood cells were washed with a physiological solution prior to preparation of the hemolysate, with no interference from plasma proteins. To achieve this, we mixed 200 µl of whole blood with 1000 µl of physiological solution, centrifuged until the red blood cells had settled, removed 1000 µl of supernatant, added another 1000 μl of physiological solution and mixed. These centrifugations and mixing steps were repeated twice. After the last centrifugation, all the supernatant was removed, and the red blood cells were treated as washed red blood cells. Then, each sample or control was diluted with hemoglobin heamolysant to obtain а concentration between 1.0 and 2.0 g/dl. To perform electrophoresis, HELENA Bioscience SAS-MX Alk Hb Kit gel was used following the factory protocol.

2.3.2 Reading electrophoresis results

Qualitative assessment; Identification of the various hemoglobin bands in the samples is carried out by visual observation of the colored gel. Fig. 2 illustrate the position of the most commonly encountered hemoglobin.

Quantitative evaluation: The percentage of each hemoglobin fraction is obtained using QUICK SCAN2000 WIN software, and read using an EPSON V700 dual lens system scanner.





Fig. 1. Map of the study area



Fig. 2. Identification of different hemoglobin bands

Most common hemoglobinopathy:

- Sickle cell trait: This heterozygous condition shows the presence of HbA, HbS and normal HbA2 in cellulose acetate. Results at acid pH reveal hemoglobin migrating to the A and S positions.
- Sickle cell disease: This homozygous state shows almost exclusively HbS, with occasional low levels of HbF.
- Hemoglobinosis S-C: This heterozygous state is characterized by the presence of HbS and HbC. Thalasso-sickle cell disease: This condition presents fractions

of HbA, HbF, HbS and HbA2. In thalassosickle cell disease β 0, HbA is absent, while in thalasso-sickle cell disease β +, HbA is present but in small amounts.

- Hemoglobinosis C-thalassemia: HbA, HbF and HbC fractions are present.
- Hemoglobinosis C: This homozygous state shows exclusively HbC.
- Thalassemia major: This condition shows HbF, HbA and HbA2 fractions.

The quantitative assessment of different detected hemoglobins was consigned in Fig. 3.



Fig. 3. Quantification of different detected hemoglobins

2.3.3 Thick smear

The thick smear is a technique for concentrating parasites on a slide from a drop of capillary blood. It is based on the principle of spreading a thin circular drop of blood, one cm in diameter, over the center of a slide.

- Place a drop of blood in the middle of a slide bearing the patient's number.
- Using the corner of a second clean slide, spread the drop over a diameter of 1 cm, turning for a few seconds.
- Leave the slides to dry, protecting them from flies and dust.
- The thick drop should be transparent.
- Staining.

Slides were stained on site with 5% diluted Giemsa stain (i.e. 5 ml pure Giemsa to 95 ml buffered distilled water) for 25-30 min. The buffered water was prepared by dissolving 1 buffer tablet in 1 liter of distilled water.

At the end of the staining time, the slides were rinsed with tap water, then dried in a microwave oven.

The dried thick drops were immediately examined and the results recorded in the parasitology register and on the clinical follow-up sheets.

Reading: Readings were taken using an optical microscope on site, with objective 100 at immersion.

Using a hand-held counter, parasites and leukocytes were counted. Counting began as soon as a parasite was observed in the field being viewed, and ended when the number of leukocytes counted reached 300. The parasite load was expressed by dividing the number of parasites per 300 leukocytes by 7500 leukocytes. We considered 7500 leukocytes to be the average number of leukocytes per mm³ of blood in a normal subject.

2.3.4 Sickle cell test procedure

Reagent preparation: 2% sodium metabisulfite solution (2 grams per 100 ml) in distilled water: dissolve 40 mg sodium metabisulfite in 2 ml distilled water or 100 mg in 5 ml distilled water.

1) Place a very small drop of blood (approx. 5 μ I) on a slide, and just beside or on top of it, place a drop approx. Four times larger (approx. 20 μ I) of reagent.

2) Mix quickly but thoroughly and aspirate about half the liquid.

3) Quickly cover with a coverslip without creating air bubbles.

4) Leave to stand for 30 minutes in a small humid chamber (protected from light).

Reading: Look for sickling under a microscope, objective 40. If negative, re-examine 2 h later. If still negative, preferably polish the slide with nail varnish (or paraffin), store in a humid chamber and examine 24 h later. The test is negative if the

red blood cells retain their round shape. The test is positive if the red blood cells gradually take on a sickle shape, like banana leaves with pointed ends, often serrated.

2.4 Data Analysis

Descriptive analyses were performed to characterize the sample. Relationships between hemoglobin types, genotypes and parasite density were examined using Student's t-tests, ANOVA, and linear regression analyses, using Python software.

3. RESULTS

3.1 Characteristics of the Population

This study included 875 participants with a sex ratio of 1.06 and a mean age of 14.25 years (range: 2 months to 80 years). Thick smear analysis of all participants revealed 52.91% positive and 47.09% negative, and the calculation of parasite density showed a mean of 242 p/uL (40 p/uL - 2600 p/uL). Sickle cells were presents in 59.09% and sickle cell features were present in 60%. Analysis of the genotypic distribution revealed the predominant of AA2

(39.31%) and SS (27.20%). The predominant hemoglobin types were HbA (66.17%) and HbS (29.14%). The presence of hemoglobin F was determined in 27.31% of study population (Table 1).

3.2 Hemoglobin Type Influence on Parasite Density

The geometric means of parasite density for each predominant hemoglobin type show that HbC (289.65) and HbS (291.39) have the highest parasitemia, followed by HbF (217.97) and HbA (192.36), HbA2 (76.67) having the lowest parasitemia ($p=2,28x10^{-59}$). We also performed pair-wise comparisons between hemoglobin types to determine which pairs showed the most significant differences using the Wilcoxon test with adjusted *p*-values < 0.05; the HbA vs HbS comparison revealed an adjusted ($p = 1.08 \times 10^{-52}$) and that of HbA vs HbC an adjusted (p-value = 9.86x¹⁰⁻⁵). These results show that the differences in parasite density between HbA and HbS and HbC are statistically significant, suggesting that HbS and HbC have a significant impact on the geometric mean of parasite density.

Table 1. Prevalence and distribution of parameters in the study population

Aspect	Details				
Gender Distribution	Males: 451, Females: 424				
Age Distribution (Months)	Mean: 171 months, Median: 132 months, Min: 2 months, Max: 960 months				
	25th Percentile: 30 months, 75th Percentile: 240 months				
Thick Drop Test Results	Positive: 52.91% (463/875), Negative: 47.09% (412/875)				
Parasite Density	Mean: 242 P/µl, Median: 40 P/µl, Min: 0 P/µl, Max: 2600 P/µl				
	25th Percentile: 0 P/µI, 75th Percentile: 340 P/µI				
Presence of Sickle Cells	Present: 59.09% (517/875), Not Present: 40.91% (358/875)				
Presence of Sickle Cell Traits	Present: 60.00% (525/875), Not Present: 40.00% (350/875)				
Genotype Distribution	AA2: 39.31% (344/875), SS: 27.20% (238/875), AS: 26.51% (232/875),				
	SC: 4.80% (42/875), A2S: 0.91% (8/875)				
	AA: 0.69% (6/875), AC: 0.57% (5/875)				
Predominant Hemoglobin	HbA: 66.17% (579/875), HbS: 29.14% (255/875), HbC: 3.66% (32/875),				
Types	HbF: 0.57% (5/875), HbA2: 0.46% (4/875)				
Presence of HbF	Present: 27.31% (239/875), Not Present: 72.69% (636/875)				
Gender Distribution	Males: 451, Females: 424				
Age Distribution (Months)	Mean: 171 months, Median: 132 months, Min: 2 months, Max: 960 months				
	25th Percentile: 30 months, 75th Percentile: 240 months				
Thick Drop Test Results	Positive: 52.91% (463/875), Negative: 47.09% (412/875)				
Parasite Density	Mean: 242 P/µl, Median: 40 P/µl, Min: 0 P/µl, Max: 2600 P/µl				
	25th Percentile: 0 P/µl, 75th Percentile: 340 P/µl				
Presence of Sickle Cells	Present: 59.09% (517/875), Not Present: 40.91% (358/875)				
Presence of Sickle Cell Traits	Present: 60.00% (525/875), Not Present: 40.00% (350/875)				
Genotype Distribution	AA2: 39.31% (344/875), SS: 27.20% (238/875), AS: 26.51% (232/875),				
	SC: 4.80% (42/875), A2S: 0.91% (8/875)				
	AA: 0.69% (6/875), AC: 0.57% (5/875)				
Predominant Hemoglobin	HbA: 66.17% (579/875), HbS: 29.14% (255/875), HbC: 3.66% (32/875),				
Types	HbF: 0.57% (5/875), HbA2: 0.46% (4/875)				
Presence of HbF	Present: 27.31% (239/875), Not Present: 72.69% (636/875)				

Comparison	Geometric mean parasite density	t-statistic	p-value
Sickle Cell (No vs Yes)	135.58 vs 271.46	-11.45	2.41x ¹⁰⁻²⁸
HbA2 (No vs Yes)	277.21 vs 123.59	12.10	4.03x ¹⁰⁻³¹
HbF (No vs Yes)	202.37 vs 288.57	-9.16	5.01x ¹⁰⁻¹⁸

Table 2. Geometric mean of parasite density by sickle cell, HbA2 or HbF presence or not

Table 3. Parameters influencing the geometric mean of parasite density in the studypopulation

Factor	Coefficient	Std Error	t-value	p-value	95% Confidence Interval
Intercept (const)	-360.11	130.88	-2.751	0.006	-616.98 to -103.24
Thick smear	391.12	32.05	12.205	0.000	328.23 to 454.01
Age	-0.05	0.08	-0.591	0.555	-0.21 to 0.11
Sickle cell presence (Yes)	-213.29	135.44	-1.575	0.116	-479.10 to 52.53
Drepanocytic_Trait (Yes)	224.92	173.87	1.294	0.196	-116.32 to 566.17
Hb Predominant (HbA2)	-306.34	226.98	-1.350	0.177	-751.81 to 139.14
Hb Predominant (HbC)	155.58	71.12	2.188	0.029	15.99 to 295.17
Hb Predominant (HbF)	-223.69	188.96	-1.184	0.237	-594.55 to 147.16
Hb Predominant (HbS)	-8.88	84.72	-0.105	0.917	-175.16 to 157.40
HbF Present (Yes)	74.24	85.17	0.872	0.384	-92.91 to 241.39
HbA2 Present (Yes)	-43.97	123.99	-0.355	0.723	-287.31 to 199.37

3.3 Association between Presence of HbS, HbF and HbA2 with Parasite Density

Individuals carrying the sickle cell trait (HbS) had a significantly higher geometric mean parasite density (271.46) compared to those without it (135.58), $p = 2.41 \times 10^{-28}$. Similarly, individuals carrying the HbF variant had a higher geometric mean parasite density (288.57) compared to those without it (202.37), p-value of 5.0x 10⁻¹⁸. Conversely, those with the HbA2 variant had a significantly lower geometric mean parasite density (123.59) compared to those without the variant (277.21), with a p=4.03x10⁻³¹ (Table 2).

3.4 Regression Analysis to Determine Parameters Influencing the Parasite Density

To determine the parameters influencing the geometric mean of parasite density in the study population, a regression analysis was conducted (Table 3). The analysis revealed several key factors and their impacts on parasite density. Age had a coefficient of -0.05 with a p-value of 0.555, showing no significant effect on parasite density. The sickle cell status (yes) had a coefficient of -213.29 with a p-value of 0.116, suggesting a potential reduction in parasite density, although this was not statistically significant. Hemoglobin C showed a significant positive influence (p=0.029). Other factors, such as the sickle cell trait, HbA2, HbF, and HbS did not have statistically significant impacts on

parasite density. The R-squared value of 0.284 indicates that approximately 28.4% of the variance in parasite density can be explained by the independent variables in this model.

4. DISCUSSION

Numerous studies have been carried out to understand the relationship that might exist between the different genotypes of sickle cell disease and parasite density during malaria (Farouk et al., 2024; López et al., 2010; Oleinikov et al., 2024; Seidu et al., 2023). In order to achieve this objective, it is necessary to know the prevalence of each of the two diseases. This study, carried out in Maradi region of Niger in West Africa, found that 52.91% (463/875) of the study population was positive for Plasmodium, which is in line with a confirmed incidence rate 202.5/1000 in this region (National Institute of Statistics, 2023). Hemoglobin A (HbA) is the predominant hemoglobin type in this study 66.17% (579/875), this is also high for the Ashanti district 76,6% (774/1010) (Kreuels et al., 2010), and the district of Begoro 80,6% (258/320) (Tetteh et al., 2021) in Ghana. This could be due to the age disparity of the study populations, with young people aged 3 months for Ashanti and 6 months to 15 years for Begoro, while our sample ranged in age from 2 months to 80 years. The main physiological function of hemoglobin A is to transport oxygen from the lungs to the tissues (Lukin & Ho, 2004). It is chemically composed of four subunits of hemoglobin A (alpha and beta) and it's tetragonal

symmetry is maintained by the configuration of alpha chains in contact with beta chains (Peisach et al., 1969).

The geometric mean of the parasite density related to the different hemoglobin types in this study shows that people with predominant hemoglobin type C (289.65 p/uL) and type S (291.39 p/uL) have the highest parasite densities. This could be in line with research from Kaduna, Nigeria, that indicates a high parasite density could also result from HbS's lack of protection against severe malaria (Dikwa et al., 2021); and another study carried out in vivo in the state of Yobe indicates that HbS does not prevent parasite invasion of red blood cells, substantial levels of parasitemia have been seen in HbS-containing red blood cells (Daskum & Ahmed, 2018). This is in contrast to a study that demonstrated how low oxygen level in HbScontaining red blood cells affected the parasites' ability to develop. Archer et al demonstrated that HbS polymerization is responsible for the inhibition of P. falciparum growth (Archer et al., Another study show that HbC 2018) homozygous red cells do not support malaria parasite growth, while heterozygous cells are competent, likely due to enhanced sickling of HbS-containing red cells (Friedman et al., 1979). These studies were carried out in vitro and may be different from what can be observed in vivo. In our student we have highlighted that people with hemoglobin S or C as the predominant hemoglobin have high parasitemia contrary to what is observed in vitro. It has also been shown that parasites are able to develop in erythrocytes containing HbC (Fairhurst et al., 2003). Previous research has demonstrated that the parasiteinfected HbS and HbC can be removed from circulation more quickly. HbS or HbC-containing red blood cells infected with P. falciparum cytoadhere to the capillary endothelium less well and this can contribute to the malaria pathogenesis in sickle cell disease (Fairhurst et al., 2012).

In pairwise comparisons between hemoglobin types to determine which pairs showed the most significant differences using the Wilcoxon test (HbA vs HbS and HbA vs HbC), suggest that HbS and HbC have a significant impact on parasite density. In a study carried out in Benin, a higher mean parasite density was found in SS subjects (4,320.7 ± 2,185 trophozoites/pl) than in SC subjects (1,564.4 ± 1,221 trophozoites/pl; p < 0.0001) (ZOHOUN et al., 2024). Lower parasite densities higher proportion and а of

submicroscopic *P. falciparum* infections were observed in Ghanaian children with HbAS trait while those with HbC had an increased risk of *P. malariae* infection (Danquah et al., 2010). It could be that the plasmodial species do not have the same replication property depending on the type of hemoglobin. In our study we did not identify the species types, even if it is established that in Niger there is the circulation of the five species of *Plasmodium* (Garba et al., 2024).

Unfortunately, the study showed that patients with predominantly HbF hemoglobin had a high geometric mean parasite density (288.57 p/uL). This result can be justified by the fact that the gamma chain of HbF is made up of isoleucine, which is adapted to the growth of the parasite (Immunology Division, ICMR-National Institute of Malaria Research, Dwarka, New Delhi et al., 2017; Istvan et al., 2011). Others suggest that HbF alters the display of the PfEMP1 protein on infected red blood cells, in particular the adhesion of infected cells to microvascular endothelial cells. thereby attenuating the pathogenicity of the parasite (Fairhurst et al., 2012).

In our study, people with HbA2 had a lower parasite density. HbA2 is a normal variant of hemoglobin A. It is present in low concentrations in normal human blood. Hemoglobin A2 may be increased in beta-thalassemia or in individuals who are heterozygous for the beta-thalassemia gene (Ou et al., 2011). However, it's not a factor that would influence parasite density. In a previous study also, no correlation was found between HbA2 level and parasitemia intensity (Ros et al., 1978).

Regression analysis showed that hemoglobin C has a significant positive influence (p=0.029) on parasite density compared with the other factors. This could corroborate a study conducted in south-west Mali which showed that in school-age children with HbC, during the dry season is more likely to develop the disease, leading to an increase in the number of cases and suggests that schoolchildren carrying a hemoglobin C mutation may contribute disproportionately to the seasonal resurgence of malaria in parts of West Africa where the HbC variant is common (Gonçalves et al., 2017). Conversely, a study by M. Fairhurst showed that the HbC predominant is associated with low parasite densities (Fairhurst et al., 2003). F. Verra also suggests that HbC carriers have increased immune reactivity to

malaria antigens, offering them partial immunemediated protection (Verra et al., 2007).

Confounding factors such as seasonality, age etc. are not considered in our study, which could constitute bias that should be minimized in future studies.

5. CONCLUSION

This study highlights the complex interplay between hemoglobin types and parasite density in the Maradi region of Niger. Even though it is known that some types of hemoglobin can protect against infection, our results show that people who mostly have types C and S have higher parasite densities, which is different from what we saw in vitro. The significant influence of hemoglobin C on parasite density highlights the need for further research into the mechanisms by which different hemoglobin types interact with malaria parasites. This research underscores the importance of understanding the local genetic landscape and its implications for malaria susceptibility and treatment strategies. particularly in regions where sickle cell variants are prevalent. Future studies should aim to identify the specific Plasmodium species and explore the molecular mechanisms underlying these relationships to effectively inform public health interventions.

DATA AVAILABILITY STATEMENTS

Datasets are available from the corresponding author.

DISCLAIMER

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DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) have been used during the writing for English language editing of this manuscript.

ETHICAL APPROVAL

This study was conducted according to ethical principles and received approval from the

institutional review board (IRB) of the faculty of medical sciences at the Université André Salifou Zinder (FSS-UAS), Niger. The IRB reviewed the research plan, ensuring that it adhered to ethical standards and guidelines for conducting research involving human subjects.

CONSENT

As per international standards or university standards, Participants' written consent has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

Archer, N. M., Petersen, N., Clark, M. A., Buckee, C. O., Childs, L. M., & Duraisingh, M. T. (2018). Resistance to Plasmodium falciparum in sickle cell trait erythrocytes is oxygen-dependent driven by growth inhibition. Proceedings of the National Academy of Sciences, 115(28), 7350-7355.

https://doi.org/10.1073/pnas.1804388115

- Badoum, E. S., Sermé, S. S., Yaro, J. B., Coulibaly, S. A., Kargougou, D., Diarra, A., Ouédraogo, A. Z., Malik, L., Nébié, I., Soulama, I., Ouédraogo, A., Tiono, A. B., Traoré, Y., Sirima, S. B., & Bougouma, E. C. (2019). Abnormalities of hemoalobin and glucose-6-phosphate-dehydrogenase deficiency in children with uncomplicated malaria and living in Banfora and Saponé, two different malaria settings of Burkina Faso. International Journal of Tropical Health, 1-10. Disease & https://doi.org/10.9734/ijtdh/2019/v37i3301 68
- Danquah, I., Ziniel, P., Eggelte, T. A., Ehrhardt, S., & Mockenhaupt, F. P. (2010). Influence S of haemoglobins and С on predominantly asymptomatic Plasmodium infections in northern Ghana. Transactions of the Royal Society of Tropical Medicine Hygiene, 104(11), and 713-719. https://doi.org/10.1016/j.trstmh.2010.08.00 1
- Daou, M., Alkasoume, I., Doutchi, M., Boubacar, S., Anou, M. M., Lamine, M. M., Lazoumar, R. H., Moumouni, K., Hamadou, D. Y., & Laminou, I. M. (2019). Sickle cell disease and severity of malaria. *International*

Journal of Tropical Disease & Health, 33(4), 1-6.

- https://doi.org/10.9734/IJTDH/2018/45337 (2018). Daskum, A. M., & Ahmed, M. Plasmodium falciparum malaria and hospital associated visits among patients with hemoglobin variants attending general hospital Potiskum, Yobe State, Nigeria. Journal of Infectious Diseases and Medical Microbiology, 2(2). https://www.alliedacademies.org/abstract/p lasmodium-falciparum-malaria-andhospital-associated-visits-among-patientswith-hemoglobin-variants-attendinggeneral-hospital-po-10114.html
- Dikwa, K. B., Maikaje, D. B., Yahaya, U. A., & Suleiman, A. B. (2021). Differences in parameters haematological and haemoglobin phenotypes in symptomatic and asymptomatic subiects with Plasmodium falciparum infection in parts of Kaduna metropolis, Nigeria. African Journal of Clinical and Experimental Article Microbiology, 22(3), 3. https://doi.org/10.4314/ajcem.v22i3.12
- Eleonore, N. L. E., Cumber, S. N., Charlotte, E.
 E., Lucas, E. E., Edgar, M. M. L., Nkfusai,
 C. N., Geh, M. M., Ngenge, B. M., Bede,
 F., Fomukong, N. H., Kamga, H. L. F., &
 Mbanya, D. (2020). Malaria in patients with
 sickle cell anaemia: Burden, risk factors
 and outcome at the Laquintinie hospital,
 Cameroon. *BMC Infectious Diseases*,
 20(1), 40. https://doi.org/10.1186/s12879019-4757-x
- Eridani, S. (2013). HbS protection from *P. falciparum* infection. *Journal of Advances in Medicine and Medical Research*, 790-801.
- https://doi.org/10.9734/BJMMR/2013/2427 Esoh, K., & Wonkam, A. (2021). Evolutionary history of sickle-cell mutation: Implications for global genetic medicine. *Human Molecular Genetics*, 30(R1), R119-R128.
- https://doi.org/10.1093/hmg/ddab004 Fairhurst, R. M., Bess, C. D., & Krause, M. A. (2012). Abnormal PfEMP1/knob display on *Plasmodium falciparum*-infected erythrocytes containing hemoglobin variants: Fresh insights into malaria pathogenesis and protection. *Microbes and Infection*, 14(10), 851-862. https://doi.org/10.1016/j.micinf.2012.05.00 6
- Fairhurst, R. M., Fujioka, H., Hayton, K., Collins,K. F., & Wellems, T. E. (2003). Aberrant development of *Plasmodium falciparum* in

hemoglobin CC red cells: Implications for the malaria protective effect of the homozygous state. *Blood*, 101(8), 3309-3315. https://doi.org/10.1182/blood-2002-10-3105

Farouk, A. G., Ibrahim, H. U., Yusuf, H. M., Bukar, L., Idrisa, J. A., & Usman, A. U. (2024). Malaria parasite density relationship with spleen size and fetal hemoglobin in Nigerian children with homozygous sickle cell disease. *Journal of Hematology and Allied Sciences*, 4(1), 23-31.

https://doi.org/10.25259/JHAS_32_2023

- Friedman, M. J., Roth, E. F., Nagel, R. L., & Trager, W. (1979). The role of hemoglobins C, S, and NBalt in the inhibition of malaria parasite development in vitro. *The American Journal of Tropical Medicine and Hygiene*, 28(5), 777-780. https://doi.org/10.4269/ajtmh.1979.28.777
- Garba, M. N., M. Moustapha, L., Sow, D., Karimoun, A., Issa, I., Sanoussi, M. K., Diallo, M. A., Doutchi, M., Diongue, K., Ibrahim, M. L., Ndiaye, D., & Badiane, A. S. (2024). Circulation of non-falciparum species in Niger: Implications for malaria diagnosis. Open Forum Infectious Diseases, 11(9), ofae474. https://doi.org/10.1093/ofid/ofae474
- Gonçalves, B. P., Sagara, I., Coulibaly, M., Wu, Y., Assadou, M. H., Guindo, A., Ellis, R. D., Diakite, M., Gabriel, E., Prevots, D. R., Doumbo, O. K., & Duffy, P. E. (2017). Hemoglobin variants shape the distribution of malaria parasites in human populations and their transmission potential. *Scientific Reports*, 7(1), 14267. https://doi.org/10.1038/s41598-017-14627-V
- Immunology Division, ICMR-National Institute of Malaria Research, Dwarka, New Delhi, Present Address: Regional Medical Research Centre, Belagavi, Nehru Nagar, National Highway No.4, Belagavi-590010, Karnataka, India, Awasthi, V., Chattopadhyay, D., ICMR Virus Unit, Calcutta, ID & BG Hospital, GB 4, Beliaghata, Kolkata, India, Das, J., & Immunology Division, **ICMR-National** Institute of Malaria Research, Dwarka, New Delhi; Present Address: Regional Medical Research Centre, Belagavi, Nehru Nagar, National Highway No.4, Belagavi-590010, Karnataka, India. (2017). Potential hemoglobin A/F role in clinical malaria.

Bioinformation, 13(08), 269-273. https://doi.org/10.6026/97320630013269

- Istvan, E. S., Dharia, N. V., Bopp, S. E., Gluzman, I., Winzeler, E. A., & Goldberg, D. E. (2011). Validation of isoleucine utilization targets in *Plasmodium falciparum. Proceedings of the National Academy of Sciences*, 108(4), 1627-1632. https://doi.org/10.1073/pnas.1011560108
- Kreuels, B., Kreuzberg, C., Kobbe, R., Ayim-Akonor, M., Apiah-Thompson, P., Thompson, B., Ehmen, C., Adjei, S., Langefeld, I., Adjei, O., & May, J. (2010).
 Differing effects of HbS and HbC traits on uncomplicated falciparum malaria, anemia, and child growth. *Blood*, 115(22), 4551-4558. https://doi.org/10.1182/blood-2009-09-241844
- Lamptey, H., Seidu, Z., Lopez-Perez, M., Kyei-Baafour, E., Hviid, L., Adjei, G. O., & Ofori, М F. (2023). Impact of haemoglobinopathies on asymptomatic Plasmodium falciparum infection and naturallv acquired immunity among children in Northern Ghana. Frontiers in Hematology, 2. https://doi.org/10.3389/frhem.2023.115013 4
- López, C., Saravia, C., Gomez, A., Hoebeke, J., & Patarroyo, M. A. (2010). Mechanisms of genetically-based resistance to malaria. *Gene*, 467(1-2), 1-12. https://doi.org/10.1016/j.gene.2010.07.008
- Lukin, J., & Ho, C. (2004). The structure–function relationship of hemoglobin in solution at atomic resolution. *Chemical Reviews*, 104(3), 1219-1230. https://doi.org/10.1002/CHIN.200421297
- Makani, J., Williams, T. N., & Marsh, K. (2007). Sickle cell disease in Africa: Burden and research priorities. *Annals of Tropical Medicine & Parasitology*, 101(1), 3-14. https://doi.org/10.1179/136485907X15463 8
- Malam-Abdou, B., Brah, S., Mahamadou, S., Maïga, D. A., Djibrilla, A., Daou, M., Adehossi, E., & Daouda, H. (2016). Les hémoglobinopathies au Niger : Analyse de 6532 électrophorèses réalisées au laboratoire de biochimie de la Faculté des sciences de la santé de Niamey. *Health Sciences and Disease*, 17(3), Article 3. https://doi.org/10.5281/hsd.v17i3.680
- Modiano, D., Bancone, G., Ciminelli, B. M., Pompei, F., Blot, I., Simpore, J., & Modiano, G. (2007). Haemoglobin S and haemoglobin C: "Quick but costly"

versus "slow but gratis" genetic adaptations to *Plasmodium falciparum* malaria. *Human Molecular Genetics*, 17(6), 789-799.

https://doi.org/10.1093/hmg/ddm350

- National Institute of Statistics. (2023, novembre 24). Statistical Directories. Système National d'Information Sanitaire. https://snis.ne/annuaires-statistiques/
- Oleinikov, A. V., Seidu, Z., Oleinikov, I. V., Tetteh, M., Lamptey, H., Ofori, M. F., Hviid, L., & Lopez-Perez, M. (2024). Profiling the Plasmodium falciparum erythrocyte membrane protein 1-specific G immunoglobulin response among Ghanaian children with hemoglobin S and C. The Journal of Infectious Diseases, 229(1). 203-213. https://doi.org/10.1093/infdis/jiad438
- World Health Organization (WHO). (2023). World malaria report 2023. World Health Organization.

https://iris.who.int/handle/10665/374472

- Ou, Z., Li, Q., Liu, W., & Sun, X. (2011). Elevated hemoglobin A2 as a marker for βthalassemia trait in pregnant women. The Tohoku Journal of Experimental Medicine, 223(3), 223-226. https://doi.org/10.1620/tjem.223.223
- Oxendine Harp, K., Bashi, A., Botchway, F., Addo-Gyan, D., Tetteh-Tsifoanya, M., Lamptey, A., Djameh, G., Iqbal, S. A., Lekpor, C., Banerjee, S., Wilson, M. D., Dei-Adomakoh, Y., Adjei, A. A., Stiles, J. K., & Driss, A. (2023). Sickle cell hemoglobin genotypes affect malaria parasite growth and correlate with exosomal miR-451a and let-7i-5p levels. *International Journal of Molecular Sciences*, 24(8), 7546. https://doi.org/10.3390/ijms24087546
- Pasvol, G. (2010). Protective hemoglobinopathies and *Plasmodium falciparum* transmission. *Nature Genetics*, 42(4), 284-285. https://doi.org/10.1038/ng0410-284
- Peisach, J., Blumberg, W. E., Wittenberg, B., Wittenberg, J., & Kampa, L. (1969). Hemoglobin A: An electron paramagnetic resonance study of the effects of interchain contacts on the heme symmetry of highspin and low-spin derivatives of ferric alpha chains. *Proceedings of the National Academy of Sciences of the United States of America*, 63(3), 934-939. https://doi.org/10.1073/PNAS.63.3.934
- Piel, F. B., Hay, S. I., Gupta, S., Weatherall, D. J., & Williams, T. N. (2013). Global burden of

sickle cell anaemia in children under five, 2010-2050: Modelling based on demographics, excess mortality, and interventions. *PLOS Medicine*, 10(7), e1001484.

https://doi.org/10.1371/journal.pmed.10014 84

- Ros, G. V., Moors, A., Vlieger, M. de, & Groof, E. de. (1978). Hemoglobin A2 levels in malaria patients. https://doi.org/10.4269/aitmh.1978.27.659
- Seidu, Z., Ofori, M. F., Hviid, L., & Lopez-Perez, M. (2023). Impact of sickle cell trait hemoglobin in *Plasmodium falciparum*infected erythrocytes (p. 2023.07.28.551025). *bioRxiv.* https://doi.org/10.1101/2023.07.28.551025
- Taylor, S. M., Parobek, C. M., & Fairhurst, R. M. (2012). Haemoglobinopathies and the clinical epidemiology of malaria: A systematic review and meta-analysis. *The Lancet Infectious Diseases*, 12(6), 457-468. https://doi.org/10.1016/S1473-3099(12)70055-5
- Tetteh, M., Addai-Mensah, O., Siedu, Z., Kyei-Baafour, E., Lamptey, H., Williams, J., Kupeh, E., Egbi, G., Kwayie, A. B., Abbam, G., Afrifah, D. A., Debrah, A., & Ofori, M.

(2021). Acute phase responses vary between children of HbAS and HbAA genotypes during *Plasmodium falciparum* infection. *Journal of Inflammation Research*, 14, 1415-1426. https://doi.org/10.2147/JIR.S301465

- Verra, F., Simpore, J., Warimwe, G. M., Tetteh, K. K., Howard, T., Osier, F. H. A., Bancone, G., Avellino, P., Blot, I., Fegan, G., Bull, P. C., Williams, T. N., Conway, D. J., Marsh, K., & Modiano, D. (2007). Haemoglobin C and S role in acquired immunity against *Plasmodium falciparum* malaria. *PLOS ONE*, 2(10), e978. https://doi.org/10.1371/journal.pone.00009 78
- Zohoun, A. G. C., Bagloagbodande, T., Adanho, A., Massi, R., Houssou, B., Orou Guiwa, G. G., Déhoumon, J., Mehou, J., Anani, L., Vovor, A., & Kindegazard, D. (2024). Anomalies de l'hémogramme dans l'association drépanocytose et paludisme chez l'adulte en hématologie clinique au CNHU-HKM Cotonou (Bénin). de Médecine Tropicale Santé et 4(1), mtsi.v4i1.2024.404. Internationale, https://doi.org/10.48327/mtsi.v4i1.2024.40 4

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