Open Access Research Journal of
Life Sciences
Journals home page: https://oarjpublication/journals/oarjls/
ISSN: 2783-025X (Online)

OARJ OPEN ACCESS RESEARCH JOURNALS

(RESEARCH ARTICLE)

Check for updates

Relationship between malaria parasitemia and haemoglobin variants in patients attending Rivers State University Teaching Hospital, Port Harcourt, Rivers State, Nigeria

LeBari Barine Gboeloh [1,*], Edith Wordu [1] and Appollus Ebenezer Josiah [2]

[1] *Department of Biology, Ignatius Ajuru University of education, Port Harcourt, Rivers State, Nigeria.*
[2] *Department of Paediatrics, Rivers State University Teaching Hospital, Port Harcourt, Rivers State, Nigeria.*

Open Access Research Journal of Life Sciences, 2022, 04(01), 016–026

Publication history: Received on 21 June 2022; revised on 24 July 2022; accepted on 26 July 2022

Article DOI: https://doi.org/10.53022/oarjls.2022.4.1.0057

Abstract

Malaria parasitemia and haemoglobin variants in patients attending Rivers State University Teaching Hospital, Port Harcourt, Rivers State, Nigeria was investigated. Blood samples from 385 patients attending the hospital were collected using sterile syringes into well labelled specimen bottles containing ethylene di-amine tetra acetate (EDTA). The genotype was determined using standard alkaline cellulose acetate electrophoretic technique while standard method was used to determine malaria parasitaemia. Out of the 385 patients examined for haemoglobin variants, the frequency of the genotype was 239(62.1%), 127(33%), 17(4.4%) and 1(0.3%) for HbAA, HbAS, HbSS and HbAC respectively. Malaria parasitemia in HbAA, HbAS, HbSS and HbAC was 170(64.2%), 82(30.9%), 13(4.9%) and 1(0%) respectively. HbAA was significantly ($P<0.005$) susceptible to malaria than other genotypes. The prevalence of malaria in HbAS 82(30.9%), HbSS 13(4.9%) and HbAC 1(0%) also show significance difference ($P<0.05$). Patience below the age of 6years with HbS gene had the highest malaria prevalence (21%). Malaria prevalence among males 7(53.8%) and females 6(46.2%) with sickle cell anaemia SCA had no significance difference ($p>0.05$). Sickle hemoglobin (HbS) gene protection against malaria found in heterozygous hemoglobin (HbAS) was also recorded in patients with homozygous (HbSS) gene. Prevalence of malaria is higher in patients with the normal haemoglobin (HbAA) than in patients with abnormal genes (HbAS and HbSS).

Keywords: Malaria; Parasitaemia; Haemoglobin variants; Gene; Hospital

1. Introduction

Sickle cell disease (SCD) is a group of inherited red blood cell disorders (hemoglobinopathies) that occurs when a child inherits two faulty hemoglobin genes from both parents [1]. The disease is primarily caused by mutations affecting the globin genes of hemoglobin. This group of diseases include sickle cell anemia (SCA) which is the most common, heterozygous hemoglobin sickle cell disease (HbSC) the second most frequent and sickle cell- beta-thalassemia disease [2,3,4].

Malaria is a life-threatening parasitic infection caused by protozoa belonging to the genus *Plasmodium*. It is a serious tropical disease responsible for high rate of morbidity and mortality globally, especially in sub-Saharan Africa where it is endemic. Globally, an estimated 241 million cases of malaria and 62700 deaths resulting from the infection was reported in 2020, 95% of malaria cases and 96% of malaria-related mortality occurred in Africa, and children below the age of five years account for 80% of the malaria deaths [5]. Specifically, over half of all global malaria deaths occurred in four African countries: Nigeria (31.9%), the Democratic Republic of the Congo (13.2%), the United Republic of Tanzania (4.1%) and Mozambique (3.8%) [5].

* Corresponding author: LeBari Barine Gboeloh
Department of Biology, Ignatius Ajuru University of education, Port Harcourt, Rivers State, Nigeria.

Sickle cell anemia and malaria are hereditary and parasitic diseases respectively. They are potentially lethal diseases and complement each other in a way [6]. Despite the adverse effect of the sickle hemoglobin gene, it has maintained a high frequency around the world especially in sub-Saharan Africa and indeed Nigeria. This is traced to the fact that individuals with one sickle cell allele (AS genotype) have some resistance to malaria; they do not have sickle cell anemia thus keeping the allele in the population [6,7,8]. In areas with malaria, heterozygous (AS) individuals reproduce at higher rates than those with no sickle cell alleles. On the other hand, in areas without malaria, homozygous (SS) individuals reproduce at lower rates than those without sickle cell disease (individuals with hemoglobin genotype AA). Hence, eliminating malaria will lead to a reduction in the frequency of sickle cell gene; thereby decreasing the gene pool [6].

Sickle cell anemia is particularly common among people whose ancestors come from sub-Saharan Africa, India, Saudi Arabia and Mediterranean countries [9]. Record indicates about 90% global population living with Sickle cell disease (SCD) are found in three countries: Nigeria, India, and the Democratic Republic of Congo [2,10,11,12]. An estimated 10%-45% of individuals with the sickle cell trait are found in sub-Sharan Africa and Nigeria and Ghana have an estimated prevalence of 30% and 15% respectively [13,14].

According to estimates, between 75 and 85 percent of children with Sickle Cell Disease (SCD) are born in Africa, where the death rate for children under five ranges from 50 to 80 percent [10]. Sickle cell anemia affects around 20 out of every 1000 live births in Nigeria, where 24 percent of the population carries the mutant gene. This means that every year, about 150,000 infants are born with SCA in Nigeria alone [15].

The relationship between the mutant gene and malaria have been reported by many researchers [16, 17, 18,19,20,21] and the infection has been implicated as a major cause of morbidity and mortality in patients with sickle cell anemia (SCA) [6]. There is more convincing indication to suggest that patients with SCA are protected from malaria in terms of lower malaria prevalence and parasite density among sickle cell patients [22,23,24,25]. However, in people with the homozyzous mutant gene (HbSS), the effect of fever, vomiting, diarrhea and anemia can provoke a sickle crisis that can outweigh any beneficial effect against malaria, conferred by the mutant gene, hence those registered in hospitals are placed on malaria prophylaxis [25]. Again, children with SCD are found to have low incidence of both severe malarial anemia and high-density parasitemia [26].

The infection of red blood cells by *P. falciparum* alters the cells and disease progression. In order for these parasites to evade the immune system, they create rosette which is the binding of *P. falciparum*- infected red blood cells to uninfected cells, helping the parasites to avoid immune recognition. Scientific investigations have shown that there is reduced rosette formation in HbAS individuals under deoxygenated condition [27] and reduced cytoadherence [28]. The increased sickling of these cells in deoxygenated conditions may be responsible for the impaired rosette formation and enhanced opsonization as well as clearance of parasitized HbAS red blood cells by the spleen may lead to earlier development of acquired immunity compared to that in HbAA individuals [9, 29].

The objective of this study is to assess the frequency of the mutant gene in its heterozygous and homozygous forms and the prevalence of malaria in patients with normal and abnormal gene, attending Rivers State University hospital. The results of the study could serve to influence policy direction and control strategies against malaria.

2. Materials and methods

2.1 Study Area

This study was conducted at the Rivers State University Teaching Hospital (RSUTH) formerly called Braithwaite Memorial Hospital (BMH). The teaching hospital (Fig. 1) is a major tertiary hospital in the Niger Delta with teaching and research facility. It lies within the N4^{0}47$'$ 3.4152$"$ and E7^0 0$'$ 37.3176$"$. Rivers State University Teaching Hospital is a referral hospital and provides medical services in Pediatrics, Family Medicine, Anesthesia, Pathology, Laboratories, Surgery, Radiology, Obstetrics and Gynecology, Ophthalmology, Accident Centre and Surgical/Medical Emergency. This implies the presence of modern equipment and infrastructure for adequate health care delivery; hence, a high influx of patients from within and outside the state.

2.2 Study Population

The study population included patients with sickle cell anemia (SCA)- hemoglobin genotype SS and other hemoglobin genotypes-AA and AS patients attending the pediatric and adult sections of the sickle cell anemia clinic, non- sickle cell anemia who visited their relatives and friends with sickle cell anemia (SCA) on their clinic days during the study period.

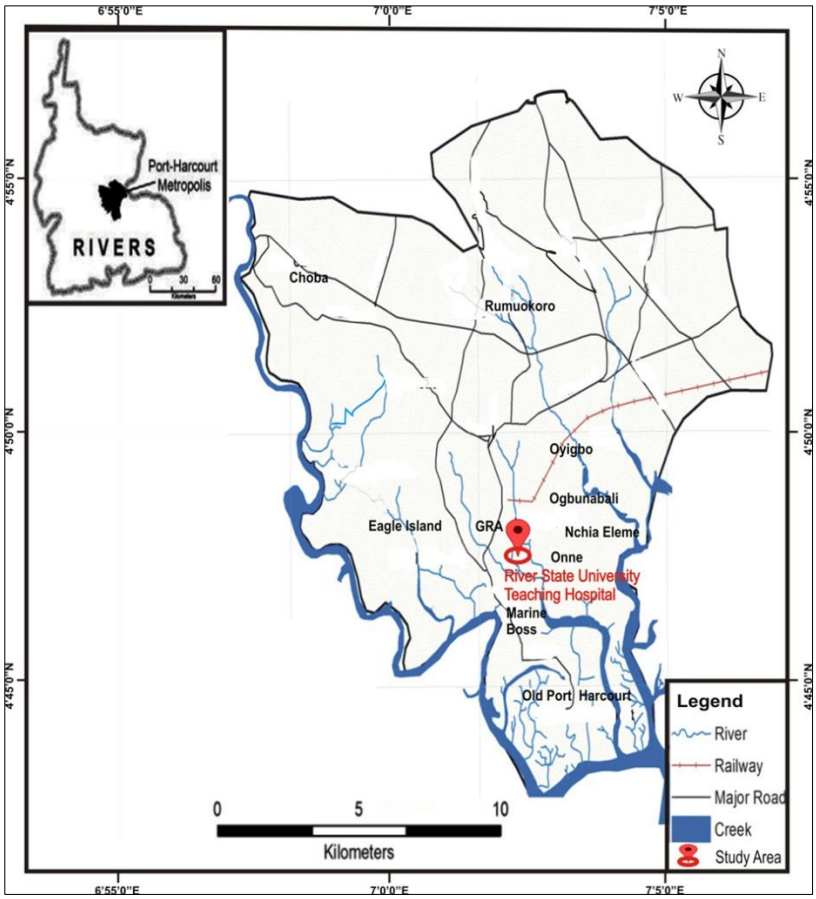

Figure 1 Rivers State University Teaching Hospital (RSUTH); (Source: Elenwo[30]; Edokpa & Nwagbara [31])

2.3 Sample Size

The sample size for this study was determined using the method of [32] with a confidence level of 95%, Z score =1.96, standard deviation of 0.5 and margin error (confidence interval) of + or - 5% =0.05.

$$\text{Sample size} = (z\ score)2 \times std\ dev \times (1 - std\ dev)/(\text{Margin error})2$$

$$= \frac{(1.96)2 \times 0.5 \times (1 - 0.5)}{(0.05)^2}$$

$$= \frac{3.8416 \times 0.5 \times 0.5}{0.0025}$$

$$= \frac{3.8416 \times 0.25}{0.0025}$$

$$= 384.16$$

Total sample size: 385 samples.

2.4 Questionnaire

Self-structured questionnaire to collect information on age, gender, educational status, occupation and knowledge of genotype was produced and distributed to the participants. Questionnaires for children/minors/wards less than 10 years were filled by their parents on their behalf.

2.5 Collection of blood samples

Blood samples for this study were collected at the Rivers State University Teaching Hospital with the help of a laboratory technologist (Phlebotomist). About 2ml of blood was collected from each subject by standard vein puncture using a 2ml

syringe, into well labeled sterile specimen bottles containing ethylenediaminetetraacetic acid (EDTA) anticoagulant. The blood samples were used to determine the hemoglobin variants (genotype) of the participants using the method of [33] and to prepare thin and thick blood smears for microscopy using the method of [34]. Each prepared blood films was carefully observed under the light microscope using X100 objective lens with immersion oil.

Hemoglobin variants of patients without knowledge of their genotype were determined using cellulose acetate electrophoresis method described by [33]. Cellulose acetate electrophoresis works with the principle that hemoglobin is a negatively charged molecule at alkaline pH 8.6 and will migrate towards the anode (+) when subjected to electric current. Different types of hemoglobin have different migration rates depending on their net negative charge. The buffer used determines the charge carried by the hemoglobin. Hemolysate is the product resulting from the lysis of red blood cells. To prepare the hemolysate, the anticoagulant blood samples were centrifuged at 2500 revolutions per minute (rpm) for five minutes in a bench centrifuge (Ocean Med+ England, model 800D). The supernatant plasma was discarded and packed cells were washed three times with large volume of saline. After the final washing, equal volume of distilled water, one quarter (1/4) volume of toluene and a drop of 3% potassium cyanide were added to the red cells and mixed properly to lyse them [33].

2.6 Electrophoresis

The buffer (Tris-acetate-borate buffer) was poured into the electrophoresis chamber. Cellulose acetate paper/membrane was cut in 40mm by 60mm rectangular size and placed inside the electrophoresis tank, with the shiny side down to soak in the buffer solution for 20 minutes. The buffer-soaked cellulose acetate paper was brought out using a stainless blunt tip forceps and placed between two layers of blotting papers to extract excess buffer from it but not to wipe dry. Using an applicator stick, 0.5ml of the already prepared hemolysate samples and already known heterozygous hemoglobin genotype AS control were applied on the cellulose acetate paper. The loaded acetate paper was immediately placed in the electrophoresis tank using the forceps. The tank was connected to the power supply and switched on to allow current run for 15 – 20 minutes. The result of hemoglobin variant distribution was read and recorded immediately The control was used to match the different hemoglobin variants according to their migration on the cellulose acetate paper as electric current was passed through it [33]. The results were graded into HbAA, HbAS, HbSS and HbAC.

2.7 Determination of Parasitemia

The level of parasitemia in each blood smear was ascertained using the malaria parasite 'plus system' count as described by [35]. Malaria parasite count of 1 to 10 parasites per 100 oil-immersion microscopic thick film fields were recorded as + (one plus) for scanty or low parasitemia; 11 to 100 parasites per 100 oil-immersion microscopic thick film fields were recorded as ++ (two pluses) for moderate parasitemia and 1 to 10 parasites per single oil-immersion microscopic thick film field were recorded as +++ (three pluses) for severe parasitemia [35,36]. The overall prevalence of malaria in the study population was calculated in percentage and the prevalence of malaria with regards to the different genotypes recorded were also calculated and analyzed.

2.8 Statistical Analysis

All data was checked for consistency before entry onto a database. Analysis of variance (ANOVA) statistical tool was used for analyzing the trend of malaria parasites occurring in different genotypes of samples investigated. Students' T-test was used to analyze malaria parasitemia between homozygous (HbSS) and heterozygous (HbAS) genotypes.

2.9 Ethical Clearance

Ethical clearance for this study was approved by Rivers State Health Research Ethics Committee, Rivers State Hospitals Management.

3. Results and discussion

3.1 Overall Prevalence of Malaria in relation to Genotype

The results indicated that out of 385 persons examined for the presence of malaria parasite in relation to genotype, an overall prevalence of 265(68.8%) was recorded. Of the 265 infected persons, 170 (64.2%), 82 (30.9%) and 13 (4.9%) were AA, AS and SS respectively (Table 1). The results showed a significant relationship ($P< 0.05$) and marked difference in the prevalence of malaria in persons with the three major genotypes (AA, AS and SS). Severe parasitaemia (+++) was recorded in AA (73.7%), followed by AS(21.1%) and SS (5.3%). Similarly, moderate parasitaemia (++) was observed in AA (70.4%), followed by AS (27.5%) and SS (2.0%) while scanty parasitaemia (+) was found in AA (58.8%), followed by AS (34.5%) and SS (6.8%) (Table 1).

Table 1 Overall Prevalence of Malaria in relation to Genotype

Genotype	No. Positive for malaria (%)					
	+	++	+++	Total Positive(%)	No. Negative(%)	Total
AA	87(58.8)	69(70.4)	14(73.7)	170(64.2)	69(57.5)	239(62.1)
AS	51(34.5)	27(27.5)	4(21.1)	82(30.9)	45(37.5)	127(33.0)
SS	10(6.8)	2(2.0)	1(5.3)	13(4.9)	4(3.3)	17(4.4)
AC	0 (0.0)	0 (0.0)	0 (0.0)	0(0.0)	1(0.8)	1(0.3)
No. Genotype	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.8)	1(0.3)
TOTAL	148(38.4)	98(25.5)	19(4.9)	265(68.8)	120(31.2)	385(100)

AA=Normal adult haemoglobin genotype. AS=Heterozygous haemoglobin A and S; SS= Homozygous haemoglobin S genotype. AC= Heterozygous haemoglobin A and C; + = Scanty infection (1-10 trophozoites in 100 microscopic field); ++ = Moderate infection (11- 100 trophozoites in 100 microscopic field); +++ = Severe infection (1-10 trophozoites per microscopic thick field)

3.2 Prevalence of Malaria between Homozygous-Sickle Cell Anemia (HbSS) and Heterozygous-Sickle Cell Trait (HbAS) Sickle Hemoglobin Gene Subjects

Result of homozygous sickle hemoglobin genotype (SS) and the heterozygous sickle hemoglobin genotype (AS) indicated that out of the 144 that tested positive for haemoglobin S (HbS) gene, 127 (88.2%) and 17 (11.8%) were AS and SS genotype respectively. The prevalence of malaria in HbAS and HbSS combined population was 95 (65.9%) while the prevalence of malaria among HbAS and HbSS were 82 (64.6%) and 13 (76.5%) respectively (Table 2). Out of the 127 persons that tested positive for HbAS gene, 82(64.6%) had malaria, of which 4(80.0%), 27(93.1%) and 51(83.6%) had severe (+++), moderate (++) and scanty (+) parasitaemia respectively. Similarly, of the 13 persons that tested positive for HbSS, 1(20.0%), 2(6.9%) and 10(16.4%) had severe (+++), moderate (++) and scanty (+) parasitaemia respectively (Table 2). There is no statistically significant difference ($P > 0.05$) in the prevalence of malaria between both hemoglobin genotypes.

Table 2 Prevalence of malaria between homozygous (HbSS) and heterozygous (HbAS) sickle haemoglobin gene (n=144)

	No. Positive (%)			No. Negative (%)		Total (%)
Genotype	+	++	+++	Total No. Positive (%)		
AS	51 (83.6)	27 (93.1)	4 (80.0)	82 (64.6)	45 (35.4)	127 (88.2)
SS	10 (16.4)	2 (6.9)	1 (20.0)	13 (13.7)	4 (8.2)	17 (11.8)
Total	61 (42.4)	29 (20.1)	5 (3.5)	95 (65.9)	49 (34.0)	144 (100)

AS = Heterozygous Hemoglobin A and S**; SS =** Homozygous Hemoglobin S genotype**; + =** Scanty (1 – 10 trophozoites in 100 microscopic thick fields)**; ++ =** Moderate (11 – 100 trophozoites in 100 microscopic thick fields); **+++ =**Severe (1 – 10 trophozoites per microscopic thick field)

3.3 Prevalence of malaria in homozygous (HbSS) and heterozygous (HbAS) sickle haemoglobin gene in relation to some demographic factors (Age and Gender)

The results also revealed that of out 144 persons that tested positive for HbS gene, 28(19.4%), 13(9.0%), 17(11.8%), 4(2.7%), 8(5.6%), 14(9.7%), 20(13.8%), 24(16.8%), 8(5.6%), 6(4.2%) and 2(1.4%) were within the age bracket of < 6years, 6-10years, 11-15years, 16-20years, 21-25years, 26-30years, 31-35years, 36-40years, 41-45years, 46-50years and >50years respectively. Out of the 95 persons infected with malaria, 21%, 7.3%, 13.8%, 2.1%, 4.2%, 10.5%, 15.8%, 14.7%, 5.3% and 5.3% belong to the age group of < 6years, 6-10years, 11-15years, 16-20years, 21-25years, 26-30years, 31-35years, 36-40years, 41-45years, 46-50years and >50years respectively. The age group of <6yrs have the highest infection rate (21.0%) while those in the age group of 10-16yrs have the least infection rate (2.1%) (Fig. 2).

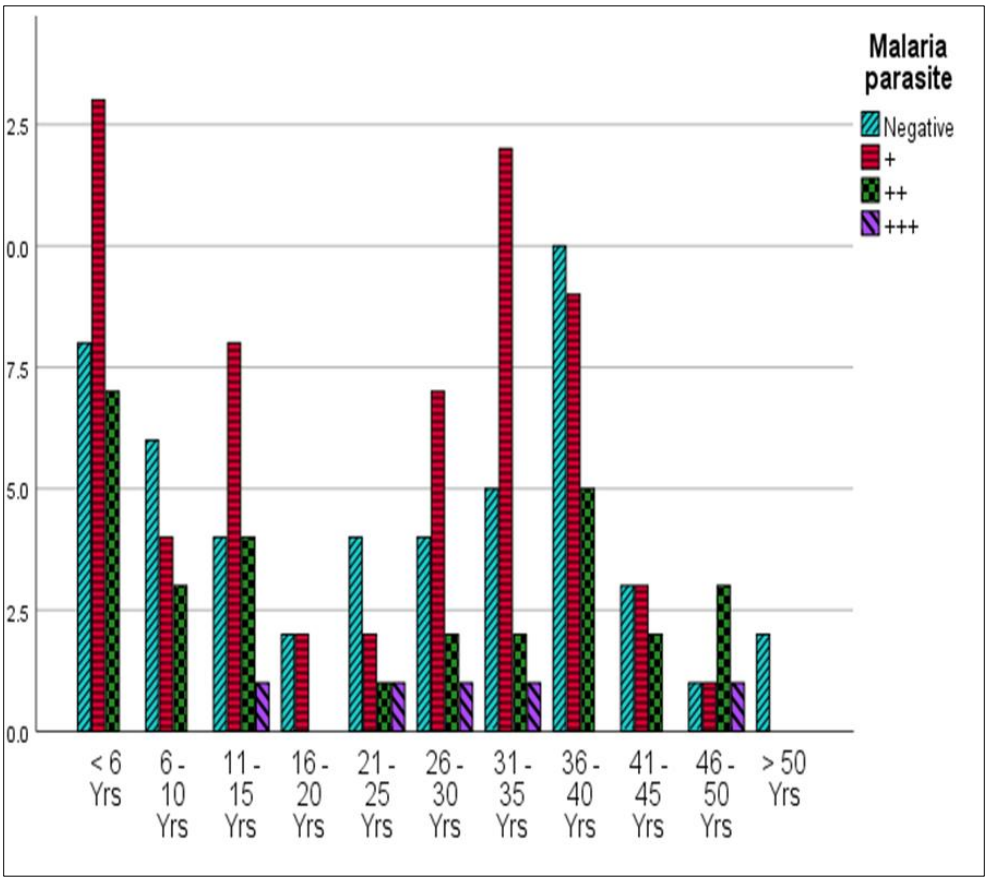

Figure 2 Prevalence of malaria among homozygous (HbSS) and heterozygous (HbAS) Sickle haemoglobin gene in relation to age

A total of 17 persons (11males and 6 females) tested positive to sickle cell anaemia (SCA). The overall malaria infection rate among sickle cell anaemia patients was 13 (76.5%). Out of the 13 infected SCA patients, 7(53.8%) and 6(46.2%) were males and females respectively (Table 3). Of the 7 infected males, 6 (85.7%), 0(%) and 1(14.3%) had scanty (+), moderate (++) and severe (+++) malaria infection respectively. Similarly, of the 6 infected patients, 4(66.7%), 2(33.3%) and 0(0%) had scanty (+), moderate (++) and severe (+++) malaria infection respectively (Table 3).

Table 3 Prevalence of malaria in sickle cell anemia (SCA) in relation to gender (n=17)

Gender	No. examined	No. Positive (%)			Total Infected (%)
		+	++	+++	
Male	11	6(85.7)	0(0.0)	1 (14.3)	7(53.8)
Female	6	4 (66.7)	2(33.3)	0 (0.0)	6 (46.2)
Total	17	10(76.9)	2(15.4)	1 (7.7)	13 (76.5)

SCA = Sickle Cell Anaemia; **+** = Scanty (1 – 10 trophozoites in 100 microscopic thick fields); **++** = Moderate (11 – 100 trophozoites in 100 microscopic thick fields); **+++** =Severe (1 – 10 trophozoites per microscopic thick field)

This study was aimed at assessing the relationship between malaria and haemoglobin S (HbS) gene in individuals carrying the Hb S gene. In the study,out of 385 patients examined, the prevalence of AA, AS, SS and AC was 62.1%, 33%, 4.4% and 1% respectively. The frequency of AA (62.1%) in this study is lower than the 73.2% recorded by [37] at Burkina Faso, the 75.73% reported by [38]) at Delta State, Nigeria but higher than the 46.7% observed by [39] among patients attending two hospitals in Benue State, Nigeria. The 33% of AS reported in this study is higher than the 19.9%, 8.2% and 29.9% recorded by [37,38,39]. In our study, SS had a frequent of 4.4% which is in agreement with the 4.37% reported by [38], higher than the 0.2% and 0% reported by Bougouma et al. [37] and Fada et al. [39] respectively but lower than 22% reported by [40] in part of Cameroon. The decreasing trend in malaria parasitemia among the

genotypes (AA:64.2%; AS: 30.9%; SS:4.9%) is in agreement with results of other studies [37,41,42]. Similar trend has been recorded in other parts of Nigeria by [38] in Benue State, [43] in Ilorin, [44] in Okada and [45] in Ogbomoso. Our result is however at variant with the record of [6,9, 47, 48, 49. The high prevalence of AA in the study may be attributed to natural selection against HbSS [38]. It is also an indication that AA is highly susceptible to malaria [25,51]. The result implies that patients with HbAS and HbSS gene enjoyed protection against malaria afforded by the presence of haemoglobin S. However, [48] reported that the protective advantage of the HbS gene is only found in its heterozygous form (HbAS).

The parasites require copious amount of oxygen and haemoglobin A to enhance its growth and development, these factors are richly provided by normal haemoglobin gene (AA) unlike what occurs with abnormal haemoglobin (HbS) which is oxygen- deficient, polymerized and poorly digested by the parasite, hence the accumulation of haemin inhibit the replication and survival of the parasites [39, 52, 53]. In normal haemoblobin A (HbA), the parasite enzyme known as malarial haem polymerase converts the harmful component of hemoglobin known as haemin (ferriprotoporphyrin) into the non-toxic chemical known as haemozoin during digestion, allowing the parasite to survive. However, because the parasite has difficulty digesting HbS, haemin builds up and prevents the parasite from reproducing and surviving in red blood cells that contain HbS [39].

The prevalence of malaria in heterozygous sickle cell (HbAS) and homozygous sickle cell (HbSS) patients was 64.5% and 8.2% respectively. This result is in accordance with the report of [39, 54, 55]. This implies that the Hb gene, in its protective effect may nt prevent infection of the blood cell by malaria parasite [56, 57]. but may prevent the progression of the disease by inhibiting development of parasite [47, 48, 58]. Among these patients, children below the age of 6years had the highest prevalent of malaria, although the parasitemia was scanty (+) than other age groups, no severe (+++) malaria was recorded. This is consistent with the record of other researchers [39,41,42]. This could be explained by the low immunity to malaria infection in children [37,51]. A condition that led to high malaria-related mortality rate among children with HbSS gene [24,40,59] .

The low malaria prevalence (4.4%) observed in patients with homozygous haemoglobin S (HbSS) or sickle cell anaemia (SCA) is in agreement with the findings of other scholars[22,23,24,25,40,60. Again, Eleonare recorded low malaria parasite density in patients with SCA. The low parasitemai recorded in this study may be as a result the protective impact of the mutant gene (HbS) causing accelerated clearance of the parasites and the hypoxia in red blood cells [40].

Malaria parasitemia between males and females in patients with homozygous sickle cell (HbSS), also called sickle cell anaemia (SCA) was not statistically significance, although males (53.8%) were numerically infected than females (46.2%). This result is contrary to the 53% and 47% recorded for females and males respectively by [61,62,63]. The high numerical prevalence of malaria in males than females may be attributed to differences in socio-economic status [39]. implementation of preventive measures against mosquito bite [64] (Ricci, 2012) and level of education [65,66].

4. Conclusion

Malaria and sickle cell anaemia are major health concern especially in sub-Saharan Africa. This study has highlighted the influence of genotype on the level of malaria parasitemia. The study revealed that malaria is still prevalent in the study area and patients with normal haemoglobin (HbAA) are more susceptible to malaria with high parasitemia than individuals with abnormal haemoglobin (HbAS and HbSS). In spite of this observation, it is necessary for individuals with abnormal haemoglobin S especially the homozygous sickle cell (HbSS)to be administered with regular antimalarial prophylaxis to reduce anemia and hemolysis caused by malaria parasite.

Compliance with ethical standards

Acknowledgments

The authors appreciate the management of Rivers State University hospital (RSUH) for approving the study, doctors and laboratory technologists for the technical assistance rendered though out the duration of the study.

Disclosure of conflict of interest

The authors disclose that there is no conflict of interest with the outcome of the study and publication of this manuscript.

Statement of ethical approval

The ethical approval for this study was obtained from the Rivers State Hospital Management Board and the Management of Rivers State University Hospital (RSUH), Port Harcourt.

Statement of informed consent

Written informed consent was also obtained from each participant.

References

[1] Emechebe GO, Onyire, NB, Orji, ML, Achigbu KI. Sickle Cell Disease in Nigeria - A Review. Journal of Dental and Medical Sciences. 2017 Jan; 16(1): 87-94.

[2] Adeyemi AA, Ladipo AO, Omolade OA, Ogbaro DD. Frequency Distribution of Hemoglobin Variants among Teenagers. British Journal of Medicine and Medical Research. 2016 Mar; 14(4): 1-5.

[3] Rezai S, Cavallo G, Gottimukkala S, Mercado R, Henderson CE. Dual Case Report of Hemoglobin SC Disease in Pregnancy. Obstetrics and Gynecology International Journal. 2016 Feb; 4(3): 00105.

[4] Serjeant GR. The natural history of sickle cell disease. Cold Spring Harb Perspect Med. 2013; 3(10): a011783.

[5] World Health Organization. Malaria Key facts [Online]., 2022 [Cited 2022 July 6. Available from: https://www.who.int/news-room/fact-sheets/detail/malaria

[6] Eridani S. Sickle cell protection from malaria. Hematol Rep. 2011 Oct; 3(3):e24.

[7] De Lange C. How sickle cell carriers fend off Malaria. [Online].,2011 [Cited 2022 July 12. Available from: https://www.newscientist.com/article/dn20450-how-sickle-cell-carriers-fend-off-malaria/

[8] Elguero E, Delicat - Leombet LM, Rougeron V, Arnathau C, Roche B, Becquart P, Gonzalez JP, Nkoghe D, Sica L, Leroy EM, Durand P, Ayala FJ, Ollomo B, Renaud F, Prugnolle F. Malaria Continues to Select for Sickle Cell Trait in Central Africa. Proceedings of National Academy of Sciences of the United States of America, 2015 May; 112(22): 7051-7054.

[9] Center for Disease Control and Prevention. Sickle Cell Disease (SCD).[Online]., 2022 [Cited July 27. Available from: https://www.cdc.gov/ncbddd/sicklecell/data.html

[10] De Baun MR, Galadanci NA, Sickle cell disease in sub-Saharan Africa. [Online]., 2022[Cited July 27. Available from: https://www.uptodate.com/contents/sickle-cell-disease-in-sub-saharan-africa/print

[11] Aygun B, Odame I A global perspective on sickle cell disease. Pediatric Blood and Cancer, 2012 April; 59 (2): 386-390.

[12] Makani J, Cox SE, Soka D, Komba AN, Oruo J, Mwamtemi H, Magesa P, Rwezaula S, Meda E, Mgaya J, Lowe B, Muturi D, Roberts DJ, Williams TN, Pallangyo, K, Kitundu J, Fegan G, Kirkham FJ, March K, Newton CR. Mortality in Sickle Cell Anemia in Africa: A Prospective Cohort Study in Tanzania. PLoS ONE, 2011 February; 6(2): e14699.

[13] Onuigwe FU, Samuel B, Erhabor O, Abdulrahaman Y. Prevalence of Some Hemoglobin Variants among Students of Usman Danfodiyo University, Sokoto, North Western Nigeria. International Journal of Clinical Medicine Research, 2014 Nov; 1(4):166-171.

[14] Therese ODM, Isah SB, Timigh GC, Daniel ET, Asongu JJ, Atanga SN, Babadoko AA. Awareness and Knowledge of Sickle Cell Disease in Rivers State, Nigeria. Texila International Journal of Nursing, 2019 Jun; 5 (2): 1-6.

[15] Faremi FA, Olawatosin OA. Quality of life of adolescents living with sickle cell anaemia in Ondo State, Nigeria. Pan African Medical Journal. 2020 April 15;35:124.

[16] Diagne I, Soares GM, Gueye A, Diagne-Gueye N, Faul N'Diaye O, Camara BN, Diouf S, Fall M. Infections in Senegalese Children and Adolescent with Sickle Cell Anemia: Epidemiological Aspects. Dakar Medical, 2000 Feb; 45(1):55-58.

[17] Ibidapo MO, Akinyanju OO. Acute Sickle Cell Syndromes in Nigerian Adults. Clinical and Laboratory Hematology, 2000 Jun; 22(3): 151-155.

[18] Faremi FA, Olawatosin OA. Quality of life of adolescents living with sickle cell anaemia in Ondo State, Nigeria. Pan African Medical Journal. 2020 Apr 15;35:124.

[19] Ambe JP, Fatunde JO, Sodeinde OO. Associated Morbidities in Children with Sickle Cell Anemia presenting with Severe Anemia in a Malarious Area. Tropical Doctors, 2001 Jan; 31(1), 26-27.

[20] Juwah AL, Nlemadim A, Kaine W. Clinical Presentation of Severe Anemia in Pediatric Patients with Sickle Cell Anemia seen in Enugu, Nigeria. American Journal of Hematology. 2003 Mar; 72 (3):185-191.

[21] Gupta NK, Gupta M. Sickle Cell Anemia with Malaria: A Rare Case Report. Indian Journal of Hematology and Blood Transfusion. 2014 Mar; 30(1):38-40.

[22] Khera G. How Sickle Cell Protects against Malaria. Scientific Animations, California. 2017 Jun; https://www.scientificanimations.com

[23] Awotua-Efebo O, Alikor EA, Nkanginieme KE. (2004). Malaria Parasite Density and Splenic Status by Ultrasonography in Stable Sickle-Cell Anemia (HbSS) Children. Nigerian Journal of Medicine. 2004 Jan-Mar; 13(1): 40-43.

[24] Kotila R, Okesola A, Makanjuola O. Asymptomatic Malaria Parasitemia in Sickle -Cell Disease Patients: How Effective is Chemoprophylaxis?. Journal of Vector Borne Diseases. 2007 April; 44(1): 52-55.

[25] Komba AN, Makani J, Sadarangani M, Abgo TA, Berkely JA, Newton CRJC, Marsh K, Williams TN. Malaria as a cause of Morbidity and Mortality in Children with Homozygous Sickle Cell Disease on the Coast of Kenya. Clinical Infectious Disease. 2009 Jul; 49(2):216-222.

[26] Makani, J., Komba, A. N., Cox, S. E., Oruo, J., Mwantemi, K., Kitundu, J., Magesa P, Rwezaula S, Meda E, Mgaya J, Pallangyo K, Okiro E, Muturi D, Newton C, Fegan G, Marsh K,Williams TN. Malaria in Patients with Sickle Cell Anemia: Burden, Risk Factors and Outcome at the Outpatient Clinic and during Hospitalization. Blood Journal. 2010 Nov; 115(2): 215-220.

[27] Aidoo M, Terlouw DJ, Kolczak MS, McElroy PD, Kuile FO, Karuiki S, Nahlen, BL, Lal AA, Udhayakumar V. (2002). Protective Effect of the Sickle Cell Gene against Malaria Morbidity and Mortality. Lancet. 2002 Apr 359(9314):311-1312.

[28] Carlson J, Helmby H, Hill AV, Brewster D, Greenworld BM, Wahlgerm M. (1990). Human Cerebral Malaria: Association with Erythrocyte Rosetting and lack of Anti-rosetting Antibodies. Lancet. Dec 336(8729):1457-1460.

[29] Cholera R, Brittain NJ, Gillrie MR, Lopera-Mesa TM, Diakité SA, Arie T, Krause MA, Guindo A, Tubman A, Fujioka H, Diallo DA, Doumbo OK, Ho M, Wellems TE, Fairhurst RM. Impaired cytoadherence of Plasmodium falciparum-infected erythrocytes containing sickle hemoglobin. Proc Natl Acad Sci U S A. 2008 Jan 22;105(3):991-6

[30] Lang PA, Kasinathan RS, Brand VB, Duranthon C, Lang KS, Foller M, Kun JFJ, Kremsner PG, Wessellborg S, Laufer S, Clement CS, Herr C, Neogel AA, Wieder T, Gulbins E, Lang F, Huber SM. Accelerated Clearance of Plasmodium Infected Erythrocytes in Sickle Cell Trait and Annexin-A7 Deficiency. Cell Physiology and Biochemistry, 2009 Nov; 24: 415-428.

[31] Elenwo EI. Socio-Economic Impact of Flooding on the Residents of Port Harcourt Metropolis in Rivers State, Nigeria. Natural Resources. 2015 Jan; 6: 1-8.

[32] Edokpa DO, Nwagbara MO. Atmospheric Stability Pattern over Port Harcourt, Nigeria. Journal of Atmospheric Pollution. 2017 Mar; 5(1):9-17.

[33] Qualtrics. Determining Sample Size: How to make sure you get the Correct Sample Size.2019 Jul; https://www.qualtrics.com

[34] Okoroiwu IL, Obeagu EI, Christain SG, Elemchukwu Q, Ochei KC. (2015). Determination of the Hemoglobin Genotype and ABO Blood Group Pattern of some Students of Imo State University, Owerri, Nigeria. International Journal of Current Research and Academic Review. 2015 Jan; 3(1): 20-27.

[35] Bejon P, Andrews L, Hunt-Cooke A, Sanderson F, Gilbert SC, Hill AVS. Thick Blood Film Examination for Plasmodium falciparum Malaria has Reduced Sensitivity and Underestimates Parasite Density. Malaria Journal. 2006 Nov; 5(104):17092336.

[36] Kloub AA. (2020). The Best Method for Estimating the Density of Malaria plasmodium Infection. Journal of Biotechnology and Immunology. 2020 May; 2(2):1-5.

[37] Cheesbrough M. Examination of blood for parasites. District Laboratory Practise in Tropical Countries.2nd ed. Cambridge University Press; 2005.

[38] Bougouma EC, Tiono AB, Ouédraogo A, Soulama I, Diarra A, Yaro JB, Ouédraogo E, Sanon S, Konaté AT, Nébié I, Watson NL, Sanza M, Dube TJ, Sirima SB (2012). Haemoglobin variants and Plasmodium falciparum malaria in children under five years of age living in a high and seasonal malaria transmission area of Burkina Faso. Malar Journal, 2012 May; 11:154.

[39] Ito EE, Egwunyenga AO, Ake JEG. Prevalence of malaria and human blood factors among patients in Ethiope East, Delta State, Nigeria. International Journal of Medicine and Biomedical Research. 2014 Dec; 3(3): 191-201.

[40] Fada DC, Obisike VU, Onah IE, Amuta EU. Assessment of malaria parasitemia and genotype of patients attending two hospitals in Benue State, Nigeria. Animal Research International, 2020 Sept; 17(2):3640-3648

[41] Eleonore NLE, Cumber SN, Charlotte EE, Lucas EE, Edgar MM, Nkfusai CN, Geh MM, Ngenge BM, Bede F, Fomukong NH, Kamga HLF, Mbanya D. Malaria in patients with sickle cell anaemia: burden, risk factors and outcome at Laquintinie hospital, Cameroon. BMC Infect Dis. 2020 Jan; 20(1):40

[42] Griffin JT, Hollingsworth TD, Reyburn H, Drakeley CJ, Riley EM, Ghani AC. Gradual acquisition of immunity to severe malaria with increasing exposure. Proceeding of the Royal Society of Biological Sciences, 2015 Feb; 282(1801): 20142657.

[43] Pant CP, Rishikesh M, Bang YH, Smith A. Progress in malaria vector control. Bulletin of World Health Organization, 59(3): 305-492.

[44] Kolawole OM, Mokuolu OA, Olukosi YA, Oloyede TO. Comparative prevalence of Plasmodium falciparum malaria in patients attending Okelele Health Centre, Okelele, Ilorin, Nigeria. Indian Journal of Health Sciences and Biomedical Research. 2017 Jan; 10(1): 57 – 62.

[45] Otajevwo FD. Prevalence of malaria parasitaemia and its association with ABO blood grouping among students of Igbinedion University Okada, Nigeria. British Journal of Medicine and Medical Research, 2013 Jan; 3(4): 1164 – 1177.

[46] Akhigbe RE, Ige SF, Adegunlola GJ, Adewumi MO, Azeez MO. Malaria, Haemoglobin Genotypes and ABO Blood Groups in Ogbomoso, Nigeria. International Journal of Tropical Medicine, 2011 Jan; 6: 73-76.

[47] Croke K, Ishengoma DS, Francis F, Makani J, Kamugisha ML, Lusingu J, Lemnge M, Larreguv H, Fink G, Mmbando BP. Relationships between sickle cell trait, malaria, and educational outcomes in Tanzania. BMC Infect Dis. 2017 Aug;17(1): 568 568 (2017).

[48] Khera G. How Sickle Cell Protects against Malaria. Scientific Animations, California. 2017 Jun; https://www.scientificanimations.com

[49] Archer NM, Peterson N, Clark MA, Buckee CO, Childs LM, Duraisingh MT. Resistance to Plasmodium falciparum in Sickle Cell trait Erythrocytes is driven by Oxygen - Dependent Growth Inhibition. Proc. Natl. Acad. Sci. USA. 2018 Jul;115(28):7350-7355

[50] Konotey-Ahulu FID. Malaria and Sickle Cell: "Protection" or "No Protection? Confusion Reigns. British Medical Journal. 2008 Oct; 337: a1875. https://www.bmj.com

[51] Williams TN, Mwangi TN, Roberts D, Alexander ND, Weatherall DJ, Wambua S, Kortok M, Snow RW, Marsh K. (2005). An Immune Basis for Malaria Protection by the Sickle Cell Trait. PLoS Medical. 2005 May; 2 (5), e128.

[52] Opara KN, Atting IA, Ukpong IG, Nwabueze AA, Inokon II. Susceptibility of Genetic Indices to falciparum Malaria in Infants and Young Children in Southern Nigeria. Pakistan Journal of Biological Sciences. 2006 Mar; 9, 452-456.

[53] Abkarian M, Massiera G, Braun-Breton C. A Novel Mechanism for Egress of Malaria Parasites from Red Blood Cells. Blood. 2011 Apr; 117(15):4118-4124.

[54] Vaidata AB, Mather MW. Mitochondrial evolution and functions in malaria parasites. Annual Review Microbiology,2009 Oct; 63:249-267.

[55] Gendrel D, Kombila M, Nardou M, Gendrel C, Djouba F, Richard-Lenoble D. Protection against Plasmodium falciparum infection in children with hemoglobin S. Pediatr Infect Dis J. 1991 Aug;10:620-621.

[56] Carnevale P, Bosseno MF, Lallemant M, Feingold J, Lissouba P, Molinier M, Mouchet J. Plasmodium falciparum malaria and sickle cell gene in the popular Republic of Congo. I. Relationship between parasitemia and sicke cell trait in Djoumouna. Ann Genet. 1981 May;24:100-104

[57] Rozenbaum M. How Sickle Cell Protects against Malaria. 2019 Jun; https://www.understandinganimalresearch.org

[58] Mc Auley CF, Webb C, Makani J, Macharia A, Uyoga S, Opi DH, Ndila C, Ngatia A, Scott JA, Marsh K,Williams T. High Mortality from Plasmodium falciparum Malaria in Children living with Sickle Cell Anemia on the Coast of Kenya. Blood. 2010 Jun; 116 (10):1663-1668

[59] Taylor SM, Parobek CM, Fairhurst RM. Hemoglobinopathies and the Clinical Epidemiology of Malaria: a Systematic Review and Meta-Analysis. Lancet Infectious Diseases. 2012 Jun; 12: 457-468.

[60] Purohit P, Mphanty PK, Patel S, Das P, Panigraphi J, Das K. Comparative study of clinical presentation and haematological indices in hoptialized sickle cell patients with severe Plasmodium malaria. J. Infect Public Health. 2018 Jun; 11(3), 321-5.

[61] Cholera R, Brittain NJ, Gillrie MR, Lopera-mesa TM, Diakite SAS, Arie T, Krause MA, Guindo A, Tubman A, Fujioka H, Diallo DA, Doumbo OKH, Wellems TE, Fairhurst RM. Impaired Cytoadherance of Plasmodium falciparum-infected Erythrocytes containing Sickle Hemoglobin. Proc. Natl. Acad. Sci. USA. 2008 Jan;105(3):991-6.

[62] Tossea SK, Adji EG, Coulibaly B, Ako BA, Coulibaly DN, Joly P, Assi SB, Toure A, Jambou R. Cross sectional study on prevalence of sickle cell alleles S and C among patients with mild malaria in Ivory Coast. BMC Res Notes.2018 Apr; 11(1):215.

[63] Sangaré A. La douleur drépanocytaire. J Panafr Douleur. Num. special. 2019 Oct; 1-12.

[64] Mick Ya PS, Olivier M, Toni KL, Augustin MM, Gray WK, Winnie SU, Robert ML, Stanislas OW, Oscar NL. Drépanocytose chez l'enfant lushois de 6 à 59 mois en phase stationnaire: épidémiologie et clinique. Pan Afr Med J. 2017 Sept; 19:1-7.

[65] Ricci F. (2012). Social implications of malaria and their relationship with poverty. Mediterranean Journal of Haematology and Infectious Diseases. 2012 Aug; 4(2): e2012048.

[66] Kolawole OM, Babatunde AS, Jimoh AA, Balogun OR, Kanu IG. Risk determinants to congenital malaria in Ilorin, Nigeria. Asian Journal of Microbiology, Biotechnology and Environmental Science, 2010 Jan; 12(2):15-22.

[67] RBM. Roll Back Malaria Partnership; Education and Malaria. Factsheet on Malaria and the SDGs.2015. http://www.rollbackmalaria.org/files/files/about/SDGs/RBMEducationFactSheet170915.pdf