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Research Article

Hemoglobin genotype variants and *Plasmodium Falciparum* malaria in children receiving postpartum care at Faith Alive Foundation Jos, Plateau State, Nigeria

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Abstract

More and more data are showing a link between hemoglobin genotypes and *Plasmodium falciparum* malaria. In order to establish the prevalence of hemoglobin genotype variants and their association with *P. falciparum* malaria in children receiving postpartum care at Faith Alive Foundation Jos, Plateau State, we study the distribution of these variants. From each sample, thick and thin blood films were created, and hemoglobin genotypes were determined using electrophoresis. Out of 172 samples examined, 131 (76.16%) were infected with *P. falciparum* malaria while 41 (23.84%) were not infected. There was no significant difference ($p > 0.05$) in the distribution of *P. falciparum* malaria in relation to hemoglobin genotypes. *P. falciparum* malaria was highest in AA with 92 (70%) and SS was the least with 12 (9%). There was no significant difference ($p > 0.05$) in th+++e prevalence of *P. falciparum* malaria in relation to age. *P. falciparum* malaria infection was highest in the age group ≥ 12 months than age group 0 - 11 months. There was no significant difference ($p > 0.05$) in the prevalence of *P. falciparum* malaria in relation to sex. The result depicted that *P. falciparum* malaria infects more males than females. There was also no significant difference ($p > 0.05$) in the prevalence of *P. falciparum* malaria in relation to months. The month of September had the highest prevalence of *P. falciparum* malaria followed by October and August respectively. It is, therefore, recommended that public health education campaigns for mothers and healthcare givers be intensified to create awareness that will lead to the reduction of human-vector contact, especially in children.

Introduction

In many poor nations, particularly in Africa, malaria is one of the most serious public health issues and a primary cause of death. It also continues to be one of the major causes of morbidity and mortality in endemic areas, mostly affecting children under the age of five [1]. Africa south of the Sahara is home to 90% of the world's malaria deaths today [2]. This is due to *Plasmodium falciparum*, the most severe of the four human malaria parasites, being the primary source of infections in Africa [3]. Additionally, the mosquito *Anopheles gambiae*, the most potent vector of malaria, is the most pervasive and challenging to eradicate in Africa. In Africa, malaria is thought to claim the lives of one million people annually, most of whom are young children under the age of five [4]. Young children who have not yet acquired immune defenses against the parasite experience the greatest death rates [5]. A small percentage of kids reportedly have a biological edge considered to partially thwart parasite growth [6]. There were 211 million cases of malaria worldwide in 2015 and an estimated 216 million cases in 2016. Between 2010 and 2016, it is estimated that the global incidence rate of malaria dropped by 18%, from 76 to 63 cases per 1000 people at risk [4]. Children under 5 years of age are one of the most vulnerable groups affected by malaria [6]. In Africa, about 285,000 children died before their fifth birthday in 2016 [4].

There are four known species of parasites that are responsible for the infection of malaria in humans, they

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include: *Plasmodium malariae, P. vivax, P. ovale, and P. falciparum*. However, the majority of malaria infections in Africa are caused by *Plasmodium falciparum* which is the most dangerous of human malarial parasites [7]. Many children who are admitted will be suffering from life-threatening complications of *Plasmodium falciparum* malaria, such as coma and convulsions (cerebral malaria), severe anemia (requiring urgent lifesaving transfusion), and rapid breathing [6]. Approximately 90% of the world's *falciparum* infections and deaths occur in sub-Saharan Africa, the latter almost entirely in children younger than 5 years of age [2].

Malaria parasites are known to respond differently to their environment in the human host such as the structure of hemoglobin [8]. The malaria parasite does not thrive well in sickle cell individuals [8]. This natural protection has made the hemoglobin S gene resilient in malaria-infested areas, particularly in Africa [6]. The protection against malaria is bestowed only on people who have sickle cell traits and have inherited just a single gene because hemoglobin S is known to interfere with the growth and reproduction of the malaria parasite [9,10]. The allele that causes sickle cell anemia imparts resistance to malaria infection [6]. However, individuals with the HbSS gene are not protected from malaria [6,11]. Therefore, this study seeks to assess the distribution of hemoglobin genotypes and their relationship to *P. falciparum* malaria in children attending the post-natal Clinic at Faith Alive Foundation (FAF), Jos Plateau State, Nigeria.

Materials and methods

Description of the study area

Plateau State is one of the 36 states in Nigeria located in the North-central region of the country. Plateau State lies between latitude: 9°10´N and longitude 9°45´E and occupies a total land area of 30,913km² (11,936.6). It has a total population of about 3,178,569 people according to the 2006 census [12]. Jos North has a total population of about 423,300 people according to the 2006 census [13]. Jos has a sharp division between the rainy season, which lasts from April to September, or May to October, and the dry season which lasts from November to April. The mean temperature of Jos is 22°C. During the Harmattan months (December to February), the mean temperature could drop to between 8 °C and 10 °C [12]. The study was carried out in Faith Alive Foundation Hospital, Jos Plateau State, a non-government, non-profitable medical and social ministry established in 1996 by Dr. Christian Isichci, and is located at No. 29 Zik's avenue, close to Rawang Pam Township Stadium Jos. It's one of the most patronized hospitals in the Jos metropolis and environs. It has facilities for diagnostic and confirmation tests for HIV/AIDS and other infections.

Study design

Purposive sampling was used to progressively enroll children who had been diagnosed with malaria and their genotypes were examined in order to assess the association between their hemoglobin genotype status and *Plasmodium falciparum* malaria.

Study population

Children between the ages of 0 and 5 who have been diagnosed with malaria parasites at the postpartum care ward make up the population size for this study. According to Cochran's [14] formula, 354 blood samples were collected based on the current malaria prevalence rate in Children 0-5 years in Plateau state which is 36% [15].

Ethical consideration

The study protocol was approved by the Ethical Committees of Faith Alive Foundation Jos, Plateau state (Ref: FAFEC/08/34/26). The approval was on the agreement that patient anonymity must be maintained, good laboratory practice/quality control ensured, and that every finding would be treated with the utmost confidentiality and for the purpose of this research only.

Exclusion criteria

Children above 5 years and children whose blood was not tested for malaria. Children whose parents/guardians did not consent or assent to the study were excluded.

Inclusion criteria

Children between ages 0-5 years who were tested for malaria and whose parents/guardians consented or assented to the study were included.

Sample collection

Blood samples were obtained from the children by venipuncture and (heel puncture for children less than 2 years). A tourniquet was tied around the upper arm in other to make the veins prominent as well as increase blood pressure in the vein. The area where the needle was introduced into the body was cleaned thoroughly with a spirit swab. The needle was inserted into the vein and 2 ml of blood was drawn into the syringe. The tourniquet was loosed before the needle was pulled out from the vein. The blood was then transferred into a sterile EDTA (Ethylenediamine tetra acetic acid) container, mixed thoroughly to avoid clotting, and then labeled.

Clinical and laboratory diagnosis of malaria parasites

Thick and thin blood films were prepared by making a blood smear with a diameter of thick 12 mm, amount of blood for a thick smear was 6 µl, the area covered by the thick blood smear was 113.14mm², amount of blood for a thin smear was 2 µl. The blood films were allowed to air dry, placed on a staining rack, and flooded with approximately 1 ml Giemsa stain for 10 minutes, it was then allowed to stand for 30 minutes, washed with water, and allowed to air dry. The films were examined under an oil immersion microscope objective (100x). Parasitaemia was determined for febrile patients who tested positive for *Plasmodium* species by counting the number of parasites (asexual forms only) against 200 white blood cells (WBC). Counting was done using hand tally counters. The number of parasites per microliter of blood was then calculated.

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Determination of hemoglobin variants

Blood samples of subjects initially screened and confirmed for malaria were subjected to a genotype test using electrophoresis following the methods described by Okoroiwu, et al. [3].

Statistical Analysis

Data obtained were expressed in simple percentages and analyzed using R Console software (Version 3.2.2). Pearson's Chi-square test was used to compare the proportion of malaria infection in relation to genotypes, age groups, gender, and months. p - values < 0.05 were considered statistically significant.

Results

Out of the 172 samples examined, 131 (76.16%) were infected with *P. falciparum* malaria while those uninfected were 41 (23.84%) as shown in Table 1. There was a significantly different (χ^2 = 47.093, df = 1, p < 0.00001) in the prevalence rate of *P. falciparum* in relation to infected and uninfected children.

The AA genotype was the highest with 92 children representing 70%, followed by AS 27(21%) and SS was the least 12 (9%) as shown in Table 2. However, there was no significant difference (χ^2 = 5.5229, df = 2, P = 0.0632) in the distribution of *P. falciparum* malaria in relation to hemoglobin genotypes (Table 2).

Also, there was no significant difference (χ^2 = 1.6022, df = 1, p = 0.2056) in the prevalence rate of *P. falciparum* malaria between age groups (Table 2). The breakdown of the results revealed that *P. falciparum* malaria infection was highest with 113 infected children representing 66% in the age group ≥ 12 months than age group 0 - 11 months with 18 (10%). There was a highly significant difference (χ^2 = 12.8, df = 1, p = 0.0003466) in the prevalence rate of *P. falciparum* malaria in relation to the three hemoglobin genotype variants in age group 0-11 months. The result depicted that AA Children in the age group 0 – 11 months were more with 16 (12%) infected than AS children with 2 (2%). However, the distribution of prevalence of *P. falciparum* malaria in relation to the two hemoglobin genotype variants showed no significant difference (χ^2 = 0.17429, df = 1, p = 0.6763) as shown in Table 2. Nevertheless, there was no significant difference (χ^2 = 4.4876, df = 2, p = 0.1061) in the distribution of the prevalence of *P. falciparum* malaria in relation to the three hemoglobin genotype variants in age group ≥ 12 months (Table 2). The AA Children within the age group ≥ 12 months had the highest 76 (58%) prevalence rate of *P. falciparum* malaria followed by AS Children 25 (19%) and SS Children was the least 12 (9%) infected (Table 2). Similarly, there was no significant difference (χ^2 = 0.48989, df = 1, p = 0.484) in the distribution of the prevalence rate of *P. falciparum* malaria in hemoglobin genotype variants in relation to sex (Table 3). More male children 71 (54%) than females 60 (46%) were infected. However, the distribution of the prevalence rate of *P. falciparum* malaria for each of the hemoglobin genotype variants in relation to sex showed no significant difference (AA: χ^2 = 0.044232, df = 1, p = 0.8334; AS: χ^2 = 2.9167, df = 1, p = 0.08767; SS: χ^2 = 0.0064484, df = 1, p = 0.936). The prevalence rate of *P. falciparum* malaria in males and females was higher in AA Children with 45 (34%) compared with AS and SS respectively (Table 3). There was no significant difference (χ^2 = 4.9866, df = 2, p = 0.08264) in the distribution of the prevalence rate of *P. falciparum* malaria in hemoglobin genotype variants in relation to months. The month of September had the highest prevalence rate of 87 infected Children representing 66%, followed by October with 30 (23%) and August had the least of 14(11%) as shown in Table 4. The distribution of the prevalence rate of *P. falciparum* malaria for each of the hemoglobin genotype variants in relation to months showed no significant difference (AA: χ^2 = 2.6726, df = 2, p = 0.2628; AS: χ^2 = 3.7846, df = 2, p = 0.1507; SS: χ^2 = 0.67708, df = 2, p = 0.7128). The result showed that the AA genotype had the highest prevalence rate of *P. falciparum* malaria in the three months studied (Table 4).

Table 1: The distribution of *Plasmodium falciparum* malaria in relation to hemoglobin genotypes.

Genotype	No. Examined	No. Infected (%)	χ^2	Df	p value
AA	117	92 (53.45)	38.368	1	0.00001
AS	42	27 (15.70)	3.4286	1	0.06408
SS	13	12 (6.98)	9.3077	1	0.002282
Total	172	131 (76.16)			
χ^2		5.5229			
df		2			
p - value		0.0632			

Discussion

The result of this study shows an overall malaria prevalence of 76.16% among children attending the Post-Natal Clinic at FAF, Jos. This indicates that there is active transmission of

Table 2: The frequency of distribution of *Plasmodium falciparum* malaria in relation to age on hemoglobin genotype variants.

Age groups (months)	No. examined			No. infected (%)									
	AA	AS	SS	AA	AS	SS	χ^2	df	p -value	Total No. examined	Total No. infected (%)	χ^2	df	p - value
0 – 11	17	3	0	16 (12)	2 (2)	-	0.17429	1	0.6763	20	18 (10)	12.8	1	0.000347
≥ 12	100	39	13	76 (58)	25 (19)	12 (9)	4.4876	2	0.1061	152	113 (66)	36.026	1	0.00001
Total	117	42	13	92 (70)	27 (21)	12 (9)				172	131 (76)			
χ^2				1.8627	1.9744×10^{-31}						1.6022			
df				1	1						1			
p - value				0.1723	1						0.2056			

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Table 3: The frequency of distribution of *Plasmodium falciparum* malaria in relation to sex on hemoglobin genotype variants.

Sex	No. examined			No. infected (%)									
	AA	AS	SS	AA	AS	SS	χ^2	df	*p* - value	Total No. examined	Total No. infected (%)	χ^2	df	*p* - value
Female	61	14	7	47 (36)	6 (5)	7 (5)	9.5873	2	0.008282	82	60 (46)	17.61	1	0.00001
Male	56	28	6	45 (34)	21 (16)	5 (4)	0.39791	2	0.8196	90	71 (54)	30.044	1	0.00001
Total	117	42	13	92 (70)	27 (21	12(9)				172	131			
χ^2				0.044232	2.9167	0.006448					0.48989			
df				1	1	1					1			
p - value				0.8334	0.0876	0.936					0.484			

Table 4: The frequency of distribution of *Plasmodium falciparum* malaria in relation to months on hemoglobin genotype variants.

Months	No. examined			No. infected (%)									
	AA	AS	SS	AA	AS	SS	χ^2	df	*p* -value	Total No. examined	Total No. infected (%)	χ^2	df	*p* -value
August	9	4	1	9 (7)	4 (3)	1 (1)	0	2	1	14	14 (11)	14	1	0.0001828
September	79	29	8	61 (47)	19 (15)	7 (5)	2.2642	2	0.3224	116	87 (66)	29	1	0.00001
October	29	9	4	22 (16)	4 (3)	4 (3)	5.0904	2	0.07846	42	30 (23)	7.7143	1	0.005479
Total	117	42	13	92	27	12				172	131			
χ^2				2.6726	3.7846	0.67708					4.9866			
df				2	2	2					2			
p -value				0.2628	0.1507	0.7128					0.08264			

malaria in the study area. The high prevalence observed might be attributed to the period of the study (August to October) which is the period of maximum rainfall in Plateau state, Nigeria. In Nigeria, a high breeding rate of the vector and high transmission index occur throughout the year, especially during the rainy season [7]. This is consistent with the report of Oparaocha [16], who obtained 88.8% during this period and Okoroiwu, et al. [3] recorded a prevalence of 76% in their investigation. Apart from the increased number of breeding sites occasioned by incessant rains, other obvious factors like ignorance, poverty, environmental conditions, poor behavioral attitudes, and inadequate planned socio-economic projects might have contributed to increased transmission [17].

The non-significant difference in the distribution of *P. falciparum* malaria in relation to hemoglobin genotype in this study could be due to the fact that all the genotypes are predisposed to *Plasmodium falciparum* malaria infection. However, the highest prevalence rate of *P. falciparum* malaria is in the AA hemoglobin genotype variant compared with AS and SS in this study (Table 2) is attributed to the high rate of oxygen consumption and a large amount of hemoglobin ingested in the peripheral blood during the stage of replication and this makes AA genotype variant more conducive for malaria parasites to strive better in it [18]. Also, we found that the degree of parasitemia was less among children with Hb-AS and Hb-SS than among those with Hb-AA. According to Daskum and Ahmed [5], the presence of sickle Hemoglobin (Hb-S) in red cells limits the development and multiplication of the parasite. Okwa (18) provided convincing evidence which is consistent with the report of Opara, et al. [19] that where *P. falciparum* is endemic, non-immune subjects who have abnormal hemoglobin (Hb-AS) have a survival advantage over subjects, who have normal hemoglobin (Hb-AA).

The non-significant difference in the distribution of the prevalence rate of *P. falciparum* malaria in relation to age groups (Table 2) was due to the fact that the parasite affects all age groups, however, the high infection rate of *P. falciparum* malaria in age group ≥ 12 months than age group 0 - 11 months could be attributed to the gradual loss of maternally derived antibodies and the development of acquired immunity [20]. Similarly, it might be due to the decreased immunity to malaria infection with advancing age after repeated infection. It has also been shown that the incidence of malaria increases as the age increases and it is highest in the first 2 years of life when maximum morbidity and mortality occur [20].

The non-significant difference in the distribution of the prevalence rate of *P. falciparum* malaria in relation to sex on hemoglobin genotype (Table 3) is associated with the fact that *P. falciparum* malaria infects all sexes. However, the highly significant difference in the infection rate between male and female children is due to that males are more exposed to the vector than females especially when the weather is hot, by moving about bare-bodied thereby exposing themselves more to malaria vector bites than the females. This is in conformity with the report of Kalu, et al. [21] who reported that males seem to be more infected than females in Umuchieze and Uturu Communities of Abia State, Nigeria. However, studies have shown that females have better immunity to parasitic diseases and this is associated with their genetic and hormonal factors [22]. Similarly, the higher prevalence rate of *P. falciparum* malaria in males than females with hemoglobin variant AA compared with AS and SS (Table 3) could be due to that *P. falciparum* strived more in AA than in AS and SS children [19]. Similarly, genotype AA is the most prevalent genotype in this part of the world and is also more prone to malaria infection than other genotypes because of the absence of any sickle cell molecules in the blood [18].

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There was a non-significant difference in the distribution of the prevalence rate *of P. falciparum* malaria in relation to months, where the highest distribution of the prevalence rate of *P. falciparum* malaria was in the month of September compared to the months of October and August (Table 4). This is because the month of September is the peak of high rainfall in the Plateau state. This agrees with the reports of WHO [7] and Oparaocha [16] who reported that in Nigeria high breeding rate of the vectors of malaria and high transmission rate occur throughout the year, especially during the rainy season [7]. The distribution in the prevalence rate of *P. falciparum* malaria for each of the hemoglobin genotype variants in relation to months in this study showed a non-significant difference. The highest prevalence rate of *P. falciparum* malaria was in the AA genotype for the three months studied (Table 4) which is an indication that infection increases as there are more rainfall and more breeding sites [21].

Conclusion

Over the years, malaria has continued to pose serious health challenges to individuals and families across the world. This study recorded a high prevalence rate of 76.16% of malaria parasitemia among children attending post-natal care at Faith Alive Foundation. The degree of parasitemia was high among children with Hb-AA and Hb-AS than among those with Hb-SS and the incidence is more common in male children than in females. In view of the high prevalence of malaria recorded in this study, it is recommended that public health education campaigns for mothers and health caregivers be given to create awareness that may lead to a reduction of vectors and control of the disease, especially in young children. Children should be treated with antimalaria drugs every three months to prevent malaria and to kill (if any) the early stage of the malaria parasite.

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