Plasmodium falciparum malaria and hospital associated visits among patients with hemoglobin variants attending general hospital Potiskum, Yobe State, Nigeria.

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Abstract

Malaria remains a major public health concern and a threat to the lives of people living in continuous malaria transmission regions of the world. While haemoglobin variants results in increased risks of morbidity due to the abnormality of haemoglobin molecules, the possession of these variants provides some degrees of acquired protections against malaria. This research was conducted to determine the common haemoglobinopathies and variation in parasitaemia among malaria infected persons in relation to hospital visits due to malaria in Potiskum Local Government Area, Yobe State, Nigeria. Venous blood was used to determine malaria parasitaemia and genotype of patients with fever related illnesses. Results obtained revealed patients with genotype AA having high parasite load, with 20 (32.79%) having moderate parasitaemia and 39 (63.93%) with low parasitaemia. However, due to low turnout of patients with sickle cell traits (SS), result obtained shows very low 18 (56.25%) to no parasitaemia 14 (43.75%) respectively. A total of 57 patients (35 (23.33%) males and 22 (14.67%) females have AS genotypes; 32 (18 (12%) males and 14 (9.33%) females with SS genotypes and 61 (31 (20.67%) males and 30(20%) females with AA genotypes. Certain mechanisms had been proved to proffer protections in patients with abnormal haemoglobin.

Keywords: Malaria, Haemoglobin, Protection, Patients

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Introduction

Haemoglobinopathies are the common severe monogenic disorders in the globe. Sickle cell diseases are mostly found in Africa. This and other forms of haemoglobin variants results in increased risks of morbidity, due to the abnormality of haemoglobin molecules that lead to hypoxia and resulted in chronic hemolysis [1]. Sickle cell has higher frequency of haemoglobin S (HbS) gene in malaria endemic regions due to a heterozygote (HbAS) that has advantage against fatal malaria. Studies reveal that the carriers of sickle cell mutation (HbAS) have an increased expression of haemoxygenase-1 (HO-1) enzyme, whose normal role is to catabolize and mitigate the cytotoxicity of free haem which is released by the degradation of haemoglobin during erythrocytic schizogony of malaria parasites. This antioxidant molecule eventually prevents severe disease symptoms [2]. It is suggested that HbAS confers protection against malaria through mechanisms that eventually reduce parasite multiplication *in vivo*. Experiments have suggested a variety of mechanisms that keep parasitaemia in low densities *in vivo* [3]. Similarly, a combination of innate, humoral and cellular immune mechanisms combats malaria infection in humans. Where exposure to episodes of malaria transmission is frequent, innate mechanisms provide some degree of protection. A clear example is the penetration of Merozoites of *P. vivax* and *P. knowlesi* to blood groups containing Duffy buffer antigens Fya and Fyb, and these antigens is lacking in most West Africans and are therefore protected from these species of Plasmodium [4].

Normal haemoglobin (HbA) has an iron containing haem ring and four globin chains made up of two-α and two-β chains in infants and adults [5]. In a foetus however, red blood cells (RBCs) contain two-α and two-γ chains, thus, termed fetal hemoglobin (HbF). Fetal hemoglobin has been reported to attract oxygen more eagerly than normal adult RBCs, which contain hemoglobin A (HbA) [6]. This high affinity to oxygen by HbF accelerates transmission of oxygen from the mother to her fetus during fetal circulation. At birth, 50-95% of the haemoglobin is HbF while the remaining is HbA. The adjustment from production of γ to β globin chains commences in the utero and results in the continuous degeneration of HbF in the fetal RBC population, to the extent that the level of HbF at birth wanes out to 5% by three months [6]. Haemoglobin F contains 2,3-diphosphoglycerate which has a low affinity for oxygen, hence making the environment inside the RBCs unfavorable for the growth of the parasite [7]. Children living in malaria endemic African regions were shown to be protected against malaria due to the presence of maternal-acquired IgG [3,6]. Research to study the relationship between *P. falciparum* malaria and HbF has been difficult due to the presence of this maternally acquired IgG [6]. However, Olaniyi and Arinola [8] reported that HbF prevents the growth of *P. falciparum*, by a mechanism suspected to involve resistance to alteration by malaria haemoglobinase and super stability of HbF.

The sickle cell trait (HbSS) is due to a point mutation on the gene coding for β-globin. Briefly, sickle cell variant of haemoglobin is produced when a single glutamate in the β-globin chain of

HbAA is substituted with valine in the 6th position of amino acid in chromosome number 11. This substitution results in polymerization of haemoglobin at low oxygen concentration, causing the haemoglobin to form rods that leads to sickling of RBC [9]. These are found at allele frequencies of 10% in many African children. Individuals with homozygous (HbSS) suffer severe microcytic anaemia that usually result in early death, while those with heterozygous (HbAS) are healthy but experience fewer episodes of both uncomplicated and severe malaria than HbAA. On the other hand however, HbC, another form of haemoglobin variant is produced when a single glutamate in the β-globin chain of HbAA is substituted with lysine in the 6th position of amino acid in chromosome number 11. HbC form crystals that decrease the plasticity of RBCs, making it rigid [9]. This is associated with reduced risk of severe *P. falciparum* malaria in some areas such as Ghana, Burkina Faso and Mali [10]. Recent statistical techniques for combining result from different studies found that no evidence of HbC reduces prevalence of complicated malaria [9].

Thalassemia is another autosomal recessive disorder of haemoglobin that results in haemolytic anaemia. It is frequently encountered in people of Mediterranean or South Asian ancestry and occurs because of a disruption of the normal 1:1 ratio of alpha and beta globin chains. There are multiple forms of thalassemias. Imbalance of alpha and beta chains results in rapid erythrocyte destruction and turnover with a chronic haemolytic anaemia. Inheritance of thalassemia mutations with haemoglobin S will produce a sickle-thalassemia disease, which is very similar to sickle cell anaemia [1]. Two forms of thalassemia; α-thalassemia and β-thalassemia exist.

α-thalassemia

It is a form of haemoglobin variant that occurs when the gene controlling the making of alpha globin is absent or missing. The reduced production of α-globin chains leads to decreased amounts of normal $\alpha_2\beta_2$ tetramers and increased amount of unpaired β-globin chains which is produced by a 3.7 Kb deletion that leaves one functional copy of duplicated α-globin genes on chromosome 16, thereby resulting in α-thalassemia [9].

However, research shows that α-thalassemia, specifically αα/αα (normal), -α/αα (heterozygous) and -α/-α (homozygous) individuals infected with *P. falciparum* isolates from Malian children show 22% reduced binding to MVECs in (-α/αα) and 43% reduced binding to micro vascular endothelial cells (MVECs) in (-α/-α) than αα/αα [3]. It is important to note that alpha thalassemia does not protect against severe malaria by reducing parasites invasion and development within RBCs or by promoting the removal of *P. falciparum* infected RBCs (iRBC) from the blood steam [11].

β-thalassemia

Unlike α-thalassemia that results from deletion, β-thalassemia is due to a mutation from any of the over 90 mutations affecting the genes coding for the β-globin chains. Lopez et al. [12] suggested that disease severity is directly related to the degree of imbalance between alpha and beta globin chains. Several epidemiological studies have suggested that β-thalassemia confers protection against malaria [1]. Investigations with normal RBC shows that monocytes preferentially phagocytose iRBC compared to uninfected RBC, and this preference is potentiated by the binding of immunoglobulin G (IgG) to iRBC and its presentation to antigen presenting cells (APCs) such as dendritic cells [3].

Additionally, polyclonal IgG from hyper immune sera binds more eagerly to α-thalassemia and β-thalassemia iRBCs than non thalassemic iRBC, suggesting that this mechanism may favorably clear iRBC containing haemoglobin variants [9].

Studies reveal that all children carry HbF in their first few months of life. Children living in malaria endemic African regions were shown to be protected against malaria, due to the presence of acquired maternal IgG [3]. Research to study the relationship between *P. falciparum* malaria and HbF has been difficult due to possession of maternally acquired IgG [3]. Fetal haemoglobin (HbF) contains a gamma polypeptide chain in the place of the beta chain of haemoglobin A. Haemoglobin F contains 2,3-diphosphoglycerate which has a low affinity for oxygen, hence making the environment inside the RBCs unfavorable for the growth of the parasite [7].

Materials and Methods

Study site

The study was conducted in Potiskum Local Government Area of Yobe State, Northeast Nigeria. Potiskum is situated at 11°43′N 11°04′E, has an area of 559 square kilometers and a population of 205,876 according to the 2006 Census [13].

Blood sample collection

Informed consent was sought from Patients reporting to General hospital Potiskum with fever and fever related illnesses to participate in this study. These were then screened for malaria as previously described [14] and blood samples were analyzed by electrophoresis to identify their genotypes.

Determination of haemoglobin variants

Blood samples of subjects initially screened and confirmed for malaria were subjected to genotype test. Briefly, an alkaline cellulose acetate was used in an alkaline buffer, Tris-EDTA Boric Acid (TEB, pH 8.4), as previously described [15]. One or two drops of distilled water were pipetted on each of the blood samples and mixed thoroughly to lyse the red blood cells. The edge of a tiny cover slip was further dipped into the mixed blood and bands were made on the cellulose acetate paper which was then placed in an electrophoresis machine with buffer water inside the machine and covered.

The tank of the electrophoresis machine was initially filled with approximately 900 ml (TEB) 250 Volts of current was applied for 5 min to the membranes to equilibrate the membranes with the buffer. The current was turned off and 8-10 µl haemolysate (10 g/µl) was then applied on each membrane at the cathodal end using a capillary tube. The electrophoresis was run for approximately 45 min to one hour until clear area between the bands is formed [15].

Results

A total of 150 patients reporting to General Hospital Potiskum with fever and fever related illnesses were screened for the

presence of malaria parasite. Patients with confirmed malaria were further analysed for their genotypes. Results obtained revealed a total of 57 patients (35 (23.33%) males and 22 (14.67%) females to have AS genotypes; 32 (18 (12%) males and 14 (9.33%) females with SS genotypes and 61 (31 (20.67%) males and 30(20%) females with AA genotypes (Table 1).

The level of parasitaemia in patients screened for malaria was determined by counting the number of infected RBCs per field view against the uninfected RBCs. However, results indicate that patients with genotype AA had high parasite load, with 20 (32.79%) having moderate (++) parasite load per field view, and 39 (63.93%) have low parasitaemia (+) while 2 (3.28%) are uninfected (-). However, due to low turnout of patients with genotype SS (32 out of 150) result obtained shows very low 18 (56.25%) (+) to no parasitaemia 14 (43.75%) (-) (Table 2).

Discussion and Conclusion

The link between sickle cell disorder and protection against severe malaria is well established, while biochemical relationship between sickle cell disease and resistance to severe malaria is unknown [2]. This study tends to identify the relationship between hospital visits by malaria infected persons and hemoglobin variants in the study area. A total of 150 patients reporting to General Hospital Potiskum with fever and fever related illnesses were screened for malaria and later analysed by electrophoresis to determine their genotypes. Result obtained revealed that patients with genotype HbAA had high parasite load when compared with HbAS and HbSS respectively. However, comparing the level of parasitaemia between patients with HbAS and HbAA genotypes, results indicate HbAS are less infected, signifying high protection. This coincides with the findings of Fairhust et al. [3], which revealed that (i) invasion of merozoite into HbAS RBCs is impaired (ii) parasite growth in HbAS RBCs is hampered due to the low oxygen tension environment that recapitulates that of post capillary venules (iii) phagocytosis of HbAS infected *P. falciparum* red blood cells (PfRBC) is enhanced at the immature ring stage of parasite development, thus reducing parasite load.

Table 1. *Gender, malaria status and genotypes of patients screened in the study area.*

Gender	MP Status				Genotype		No. (%) Screened
	-	+	++	+++			
Male	13	20	2	-	AS		35(23.33)
Female	3	18	1	-	AS		22(14.67)
Male	1	17	-	-	SS		18(12)
Female	13	1	-	-	SS		14(9.33)
Male	1	20	10	-	AA		31(20.67)
Female	1	19	10	-	AA		30(20)
TOTAL	32	95	23	0			150

Table 2. *Malaria parasite load in relation to genotype.*

Genotype	No. (%) Screened	MP Status (%)			
		-	+	++	+++
AS	57(38)	16 (28.1)	38(66.67)	3(5.26)	-
SS	32(21.33)	14(43.75)	18(56.25)	-	-
AA	61(40.67)	2(3.28%)	39(63.93)	20(32.79)	-
	150	32	95	23	0

Taylor et al. [9] further revealed that heterozygous (HbAS) individuals have reduced risk of severe *P. falciparum* malaria by about 90%. However, Gunn and Pitt, [4] reported that the ability of *P. falciparum* to invade and develop within red blood cells is considerably reduced if the sickle cell trait (HbAS) genotype is expressed.

Conversely, Ho and White [16] opined that the possession of oxidized denatured haemoglobin (hemichromes), by HbAS RBCs interfere with actin cytoskeleton and interrupt the expression of *P. falciparum* erythrocyte membrane protein 1(PfEMP-1) to the surface of infected RBCs (iRBCs). Additionally, Taylor et al. [9] reported that HbAS and HbSS genotypes does not impair with parasite invasion, due to high parasite densities seen in HbAS and HbSS RBCs infected with *P. falciparum in vivo*. Despite their inability to impair parasite invasion, parasite growth was proved to be impaired. Growth impairment was due to the ability of HbAS infected RBCs to manifest host microRNA (miR-451 and let-7i) in high amount than HbAA. The host microRNA (miR-451 and let-7i) are translocated into the parasite, fuse with parasite messenger RNA (mRNA) transcripts and inhibit the translation of enzymes critical for parasite development [9].

In vitro studies reveal that HbAS RBCs infected with *P. falciparum* show 40-55% reduced binding to Microvascular endothelial cells (MVECs) [3]. This research further observed that of the 150 patients screened for malaria, 32 (21.33%) had HbSS homozygous genotypes and were less infected with 18 (56.25%) having low parasitaemia (+) and 14 (43.75%) uninfected (-). This corresponds with the results obtained by Eridani, [17] in his study which reported the impact of the coexistence of HbF and HbS, revaeling that such patients have a relatively mild clinical malaria with near-normal haematological parameters. Moreover, 80% reduced binding to MVECs was reported in HbSS infected RBCs (iRBCs) [3].

An important mechanism of resistance offered by various haemoglobinopathies is the impairment of RBC cytoadherence: which is the binding of *P. falciparum*-infected RBCs to endothelial cells of small vessels, a fundamental event in both parasite survival and malaria pathogenesis in humans. Following early reports on the evidence for altered or decreased surface expression of the malarial protein [*P. falciparum* erythrocyte membrane protein 1 (PfEMP1)] on infected erythrocytes from individuals heterozygous for HbS. Cyrklaff et al. [18] reported that the increased oxidation of HbS affects actin cytoskeleton formation, thereby reducing delivery of PfEMP1 to the erythrocyte surface.

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