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Evaluation of the Relationship among Different Haemoglobin Genotypes, Calcium, and Membrane Potential in Patients with Malaria in Fmc, Umuahia

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Abstract: Malaria is still a major contributor to high rate of the global infectious disease-related mortality and morbidity particularly in Nigeria**.** The aim of this study is to assess the relationship between various Hemoglobin Genotypes, Calcium and Membrane Potential in malaria patients. Retrospective analysis of the results of the distribution of Malaria parasitemia among suspected cases of malaria, various Haemoglobin genotype, Calcium and Membrane Potential were conducted in Federal Medical Centre (FMC) Umuahia, Abia State. Two hundred cases were examined by Giemsa staining method using thick film. Haemoglobin genotype determination was performed by Cellulose acetate electrophoresis. Obtained data were analyzed using One-Way ANOVA. Results: One hundred and seventy (85.0%) were positive for Malaria parasite. The prevalence was 92(54.1%) and 78(46.0%) for females and males respectively and at $p<0.05$, the result obtained from the statistics is considered significant. There are high prevalence of parasitemia in AA, 80(40.0%), with genotype SS recording the least with 30(15%). The age group 27-35 years had the highest occurrence of parasitemia with (44.1%) while the least was obtained in age range 45-53 years with (2.4%). It was observed that the density of Malaria parasitemia was highest in age group 27-35 years meaning that parasite density decreases with increasing age and the Haemoglobin genotype AA had the highest Malaria density. Also, the serum Calcium and Membrane Potential were significantly reduced in Sickle cell disease (HbSS) when compared to the HbAA and HbAS individuals. This signifies low energy level in Sickle cell patients that can result to oxidative stress. This may probably indicate that serum Calcium and Membrane Potential are significantly reduced in Sickle cell patients compared with other Haemoglobin genotype with Malaria.

Keywords: haemoglobin genotypes, calcium, membrane potential, malaria umuahia.

INTRODUCTION

Human malaria is an infectious disease of world-wide distribution caused by intracellular protozoan parasites belonging to the genius Plasmodium (Uneke, 2000). Malaria is still the most life-threatening vector-borne disease globally with an estimated 409,000 deaths and 229 million cases reported in 2019 (Global Malaria Programme: WHO Global, 2020). Malaria continues to remain the most severe and complex health challenge facing the vast majority of the countries in tropical and sub-tropical regions of the world. It is one of the most predominant infectious diseases associated with under development, poverty and ignorance (Worral, *et al*., 2005).

Malaria is still a major contributor to high rate of the global infectious disease-related mortality and morbidity particularly in Africa, South-East Asia, Eastern Mediterranean regions and parts of South America (WHO, 2008). About 500 million individuals become the victims of malaria each year. It is a highly devastating parasitic disease caused by intra erythrocytic protozoa of genus plasmodium.

An estimated three million people die from malaria each year (Breman, *et al*., 2004) and five hundred million to five billion clinical episodes of the disease are recorded worldwide (Snow, *et al*., 2005). Sub-Saharan Africa bears the greatest burden with more than one hundred and fifty million cases and about one million deaths annually mostly in children under the age of five years (WHO, 2005). There were approximately 212 million malaria cases in 2015 and an estimated 429,000 malaria death globally (WHO, 2015).

In the World Malaria Report (WMR) of 2009, the World Health Organization estimated that 243 million cases of malaria occurred worldwide in 2008 and majority of the cases (85%) occurred in the African Region followed by South-East Asia (10%) and Eastern Mediterranean Region (4%) (WHO, 2009).

The infections resulting from Plasmodium falciparum if left untreated might cause kidney and brain complications and even death (Conway, 2007; Fairhurst, *et al*., 2009).

There are four species of Plasmodium that infect man: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malaria*, *Plasmodium ovale*. The differentiations of the species depend on the morphology of the parasite. Mortality and morbidity through anaemia, cerebral complications or other mechanisms are mainly associated with Plasmodium falciparum (Uneke, 2006). The arthropod hosts are females of certain species of Anopheles mosquito. Among sixty species of Anopheles mosquitoes that are vectors for malaria,

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only thirty are major epidemiological importance (Lucas, *et al*., 1998).

Advances in molecular biology and bio-informatics (Hume, *et al*., 2003) in the past decades have provided evidence that malaria has been the strongest force for evolutionary selection in the recent history of human race (Kwiatkwoski, 2005).

The presence of geographical pattern in the distribution of the sickle cell gene and its association with malaria has been documented (Weatherall, 2001). Multiple human mutations associated with survival advantage in Plasmodium falciparum have been developed. These include structural haemoglobinopathies (the HbS, HbC), quantitative haemoglobinopathies (the thalassemias) (Zaino, 1965).

In Africa, malaria was one of the major selective forces in their evolution and as a result many genes are known to confer a survival advantage. One of the best studied gene is the gene for heamoglobin S (HbS) (Modell, 1989). In malaria-endemic areas, HbAS heterozygotes has a survival advantage when compared with HbAA individuals. The selection of the deleterious gene by survival advantage for another disease is referred to as a balanced polymorphism.

The malaria hypothesis purposes a survival advantage for individuals with haemoglobin variants in the areas of endemic Plasmodium falciparum malaria *(Aarti, et al*., 2000). The inherited disorders of heamoglobin are the most common gene disorders with 7% of the world's population being carriers.

Epidemiological and in vitro support for the malaria hypothesis is best documented for the thalassemias and sickle cell haemoglobin (HbS) (Fleming, *et al*., 1979) because heterozygotes are protected against lethal effects of falciparum malaria (Angastiniotis, *et al*., 1995). Haemoglobin C and Haemoglobin E have also been found to be associated with a reduced prevalence of severe plasmodium malaria in heterozygotes (Ringelhann, *et al.*, 1976). The WHO recently recognized sickle cell disease in Africa as a problem of major importance (Makano, *et al*., 2007*).* Considering its nature, it calls for attention in malaria endemic areas.

Calcium is one of the minerals present in the blood and other body fluids. It is important for the normal physiology of life. Calcium is a mineral element (ionized salt) present in human body fluids and the blood stream. The optimum range of calcium is essential for proper physiological activities (Medlineplus, 2014). The whole body actually acts like a bioelectric organism and the electrolyte like calcium acts as a switch and energy source for our body (Spence, 1999). Calcium is essential for parasite development during the erythrocytic stage (Garcia, 1999). Plasma calcium, specifically contributes to merozoites invasion of RBCs, as well as parasite development in RBCs (Wasserman, *et al*., 1982; Gao, *et al*., 2013; Weiss, *et al.,* 2015). Cytoplasmic calcium concentration has been shown to slowly increase parasite proteases during the schizont stage and inducing merozoities egress from intracellular RBCs (Farias, *et al.,* 2005; Garg, *et al.,* 2013; Glushakova, *et al*., 2013).

Also plasma calcium is requires for host blood coagulation. Activation of blood coagulation is frequently observed in patients with malaria, which subsequently induces inflammation and severe malaria associated symptoms. In fact, the degree of coagulation activation is proportional to the severity of disease-related symptoms such as fever and disseminated intravascular coagulation (DIC) (Angchaisuksiri, 2014; Francischeti, *et al*., 2008). DIC is associated with severe outcomes and high mortality rates. During severe complicated malaria infection, the intrinsic coagulation pathway is activated by thrombin generation which is pivotal for activation of the coagulation cascade.

Also calcium imbalance and mineral disturbance were known to be common clinical manifestations in several infectious disease including malaria (Prabha, *et al*.,1998). Calcium disturbance acts as an indicator for the severity of disease because it is usually associated with severe Plasmodium falciparum and Plasmodium vivax (Jasani, *et al.,* 2012).

Hypocalcaemia usually develops, because of infection with Plasmodium (Sitprija, 2008). Calcium which is an essential nutrient for human body provides strength for teeth and bones. It plays an important role in maintenance of health and nutritional qualities (Nordin, 1997). Hypocalcaemia is a common observation during malaria infection. Decline in calcium occurs due to clinical symptoms associated with malaria like fever, high pulse rate, sweating and shivering (Golvan, 1983). Prevalence of malaria is very high in Nigeria and Plasmodium falciparum impart heavy burdens on the entire population. Calcium

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imbalance appears because of malaria and may lead to the severity of the disease.

The membrane potential is an important property of many cells and organelles. Changes in membrane potential control accompany numerous biological processes such as information transfer in neuronal network, muscle contraction and energy transduction during photosynthesis or metabolism (Joao, 2002).

Membrane potential is the difference in the electrical potential between interior and the exterior of the biological cell. The membrane potential arises primarily from interaction between the membrane and the actions of the two types of trans-membrane proteins embedded in the plasma membrane (Nnodim, *et al.*, 2014).The increased permeability of calcium ions in linked with the shift in the concentration gradient of potassium and sodium leading to lowering of the calcium ions concentration gradients across the cell membrane (Osuagwu, *et al.*, 2007).

The membrane of excitable tissue is capable of maintaining two different states: The resting state or the acting state. These two states are defined in terms of the membrane permeability of sodium and potassium. The membrane permeability is small in the resting state whereas it is large in the acting state. The membrane potential may be dependent on the state of the membrane calcium which is located in a layer of lipoproteins (Tobias, 1958). In other words, the resting state of the membrane will be the condition in which calcium ions are associated with the membrane and the acting state, the condition in which these ions are dissociated from the membrane. This concept is supported by the recent findings that permeability of potassium and sodium is remarkably increased when the membrane calcium is removed (Kimizuka, *et al.*, 1963). It is expected that the dissociation of the membrane calcium in the external solution is high and accelerated when it is low or nullified. The membrane will tend to stay in the resting state or acting state depending on the external concentration of calcium. Indeed, the excitable membrane is depolarized and often imitates action potentials spontaneously when the concentration of calcium in the external solution is reduced (Kotetsu, *et al.*, 1962). The decrease in the trans-membrane concentration gradient of sodium, potassium ions can be individually or jointly used as a biomarker of the severity of sickle cell anaemia by evaluating the change in the membrane potential.

The study "Assessment of the relationship between various hemoglobin Genotypes, Calcium and Membrane Potential in malaria patients will reveal to us the following objectives for health planning and policy making in our nation.

Malaria parasite is still a great threat to both tropical and sub-tropical African population (WHO, 2018). It has continued to remain a major disease in tropical homogenous black African population in spite all the current strategies to eradicate Malaria parasite. The assessment of various haemoglobin genotypes in relation to calcium and membrane potential in those infected with Malaria parasites, seemed to be raised in Umuahia, Abia State. This study may serve as a tool for planning and a guide for allocation of resources in care and management of patients with haemoglobin disorder and treatment of those infected with Malaria parasites.

MATERIALS AND METHODS

Study Area

The study was conducted in Federal Medical Centre Umuahia. In Nigeria, malaria is characterized by its seasonality where the peak transmission season is from October to December with second peak in June. Plasmodium falciparum is the predominant species in this area (Alemu, *et al.*, 2012). Residents often live in non-substantive accommodation and despite a scale up in preventive measures including Insecticides Treated Net (ITN) distribution, they are at risk of malaria.

Advocacy Mobilization and Pre-survey Contacts

A formal letter of introduction was obtained from the Head of Department Medical Laboratory Science of Imo State University, Owerri. The letter with the project proposal was submitted to the ethical committee of Federal Medical Centre, Umuahia. An ethical approval letter was obtained from the Hospital to collect samples from the study subjects. Informed consent was obtained from the subjects after several meetings on their clinic days.

Study Design and Period

The study is a pilot, prospective diagnostic study of malaria in individuals attending Federal Medical Centre, Umuahia, Abia State. The goal is to assess the effect of various haemoglobin genotype, calcium and Membrane potential in malaria parasite transmission. It will further hypothesize that enhanced case detection by screening asymptomatic individuals at each

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clinical visit will be of additional value in treating malaria. Both symptomatic and asymptomatic (apparently healthy individuals) will be included in the study. The study was carried out between March and August, 2021.

Recruitment of Subject

Group One

A total of one hundred (100) malaria subject being diagnosed by the physician comprise males and female between the ages of 18 and 65 years both symptomatic and asymptomatic (apparently healthy individuals) attending clinic in Federal Medical Centre, Umuahia.

Group Two

A total number of one hundred (100) apparently healthy individuals both male and female will be recruited as control subject.

Inclusion and Exclusion Criteria

Malaria patients both males and males between the ages of 18 and 65 years presenting to the hospital will be enrolled in the study. Informed consent will be obtained from participating individuals. Those who are taking or have antimalarial medication 3 weeks prior to study commencement will be excluded.

Data Collection

A case study of 200 individuals (100 malaria patients and hundred apparently healthy and asymptomatic individuals) between the ages of 18 and 65 years attending Federal Medical Centre, Umuahia. Fasting venous blood will be collected from determination of serum calcium, haemoglobin genotype, Malaria parasite while Membrane potential will be calculated using Nernst equation. The serum calcium will be estimated using AGGAPE reagent, haemoglobin genotype by haemoglobin electrophoresis and Malaria parasite by thick film using Giemsa.

Blood Collection

In all subjects, 4ml of fasting venous blood will be collected into plain, heparin and EDTA bottles. The serum will be centrifuged at 5,000g for 10 minutes. After centrifugation, red blood cells will be separated from the plasma, washed three times with physiological saline and lysed with 1.0ml of distilled, deionized water. The red cell hemolysates was stored frozen until analysis.

Biochemical and Hematological Assay

Determination of Calcium, and Membrane Potential was done by Standard Method

Principle

Calcium ion at neutral pH form with Arsenazo 111 a complex, the colour intensity is directly proportional to the concentration of calcium in the sample.

Sample Techniques

Sample technique for this study is based on convenience and the current rate of individuals presenting to the hospital with the clinical symptoms of malaria.

Statistical Analysis

The data was analysed using ANOVA to ascertain the significant Mean±SD of serum Ca, RBC- Ca and membrane potential of HbAA, Hb AS, and Hb SS in relation to malaria parasitemia

RESULTS

Table 1: Distribution of malaria parasite by age

Parameter (Age)	Total number Examined	No positive	% positive
18-26	90	72	42.4
27-35	84	75	44.1
36-44	21	19	11.2
45-53	5	4	2.4
Total	200	170	100.1

Table 1 Shows the distribution of malaria parasite by age and 170(85%) out of 200 samples were positive for malaria parasitemia. 27-35years had 75 (44.1%) of malaria parasitemia followed by 18-26 years with malaria parasitemia of 72 (42.1%) and also, 36-44years had malaria parasitemia of 19(11.2%) respectively. Result obtained revealed the prevalence rate of 40%, 30%, and 15% belonged to genotype AA, AS and SS respectively.

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Table 2: Distribution of malaria parasitemia by sex

Male	94	78	46.0
Female	106	92	54.1
Total	200	170	100.1

Table 2 shows the distribution of malaria parasitemia among various sex. Females (54.1%) were more infected than males (46..0%). At 5% level of significance (<p0.05), the result obtained was considered significant.

Table 3: Density of parasitemia in different age groups

Age group (Year)	+(%)	++(%)	+++(%)	++++(%)
18-26	32(50)	33(44)	4(20)	2(33.3)
27-35	22(34.4)	33(44)	16(64)	4(66.7)
36-44	5(7.8)	8(10.7)	4(16)	0(0)
45-53	5(7.8)	1(1.3)	0(0)	0(0)
Total	64(100)	75(100)	25(100)	6(100)

Density of parasitemia in different age groups is shown in Table 3. The highest malaria density was obtained in the age group 27-35 years. This shows that density of parasitemia decreases as age group increases.

Table 4: Density of parasitemia in relation to Haemoglobin genotype

Hb genotype	+(%)	++(%)	+++(%)	++++(%)
AA	18(28.1)	34(45.3)	22(88.0)	6(100.0)
AS	25(39.2)	32(43.0)	3(12.0)	0(0.0)
SS	21(33.0)	9(12.0)	0(0.0)	0(0.0)
Total	64(100)	75(100)	25(100)	6(100)

Table 4 shows the density of parasitemia in relation to Haemoglobin genotype. Haemoglobin AA had the highest parasite density while both Haemoglobin AS and SS had the least parasite density.

Table 5: Mean±SD of Serum Calcium, RBC- Calcium, Membrane Potential, HbAA, Hb AS, and HbSS

Hb Genotype	Membrane potential (J)	Serum Calcium (mg/dl)	Red blood cell (mg/dl)	p-value
AA	146.07±45.82	8.91±0.92	3.66±1.21	0.001
AS	110.75±46.15	7.42±0.80	4.02±1.04	0.001
SS	71.95±20.60	5.90±0.69	4.92±2.01	0.001

p-value>0.05 was considered as significant

Table 5 shows that serum level was significantly reduced in Sickle cell disease (HbSS) when compared with the HbAA individuals. Membrane Potential was also significantly reduced in HbSS when compared with HbAA individuals.

DISCUSSION

Analysis of data collected from this study shows that malaria has continued to remain a major disease in tropical homogenous black African population. This infection is associated with great morbidity and mortality since the discovery of this infection many decades ago; it is still a great threat to both tropical and sub-tropical Africa.

In this study, there was higher rate of malaria parasitemia in this locality. Result obtained revealed the high prevalence rate belonged to genotype AA followed by genotype AS and the least malaria parasitemia was found in genotype SS. The higher prevalence in genotype AA is consistent with the earlier work of Adefioye, (2007) and Alaribe, *et al*., (1998) who reported that subjects with AA had the highest incidence of malaria parasitemia with the prevalence of 78.8% and 85% respectively. There are many hypothesis for the higher prevalence rate among genotype AA for instance, Pasvol, (1980) stated that the development of malaria parasite in blood requires adequate oxygen supply which is abundant in genotype AA against low oxygen tension obtained in AS or SS genotype hence, higher prevalence is expected in AA homozygous. The result also in agreement with the report of Allison, (1945) who

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reported that persons with sickle cell trait developed malaria less often and less severe than those without the trait.

This result contradicts that reported by Fleming, *et al*., (1979) who stated that the declining prevalence of the sickle cell trait with age in hospital admissions could imply an increased mortality of the trait or increased fitness in both malaria and non-malaria areas. The result also contradicts the work of Colbourno, *et al.*, (1956) who reported lower parasite rate and densities in children of all ages with the sickle cell trait but the protective effect was confined to children under one year.

Meanwhile, no confirmed evidence on the mechanisms by which the haemoglobin genotype AS protect against severe malaria has yet been documented (Rachanee, *et al*., 1993). HbAA has more membrane Potential when compared with HbAS and HbSS. HbSS has low energy and can be linked to low membrane potential. The enzyme has low membrane Potential and could be linked to oxidative stress.

It was observed that the level of membrane potential was significantly reduced in Sickle cell disease (HbSS) when compared with the Haemoglobin AA (HbAA) individuals. This is consistent with the work of Ibe, *et al*., (2009).

Membrane Potential was significantly decreased ($p<0.05$) in Sickle cell disease (HbSS) when compared with Haemoglobin AA individuals (HbAA). Also, the decrease in Membrane Potential has followed a systematic style in different Haemoglobin genotype: HbAA, HbAS, HbSS. The Membrane Potential translates into energy and this implies that the energy in HbSS is very low. The low level of energy in HbSS is linked to their frailty and weakness among sickle cell patients. So, much there is a strong link between the depleted membrane potential and sickle cell intensity. This is in agreement with the work of Osuagwu, *et al*., (2009).

Therefore, reduction in calcium level can lead to decrease membrane potential. It is pertinent to sensitize individuals especially the Sickle cell patients to avoid dehydration to prevent decrease in Membrane Potential which can cause sickling.

CONCLUSION

Sequel to the result of this research, it has been established that genotype AA individual are more susceptible to malaria parasite because the development of malaria parasite in blood require adequate oxygen supply which is abundant in genotype AA against low oxygen tension obtained in AS or SS genotype.

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