# PREVALENCE OF MALARIA AMONG PATIENTS WITH SICKLE CELL TRAIT IN EKITI STATE

BY

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## CERTIFICATION

I certify that this project work was carried out by OJOBANIKAN ADEMOLA OLUWASEUN with matric no mcb/11/0341 of the Department of Microbiology, Faculty of science, Federal university of Oye Ekiti, Ekiti state.

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## DEDICATION

This project work is dedicated to God almighty, for his mercy, favour and grace throughout the course of doing this project work.

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## ABSTRACT

This study was carried out to determine the prevalence of malaria parasite among patients with sickle cell trait in Ekiti state. A total of 20 samples were collected from different hospitals and clinics. The samples were collected randomly and sodium metabilsulphite was employed to confirm the sickling trait in the samples and thereafter the malaria parasite kit was used to test for the prevalence of malaria in the samples. Eight (8) persons who tested positive for sickle cell trait were tested for malaria parasites. The prevalence of the malaria parasite among patients with sickle cell trait was 2 of 8 (25%), and that of patients without sickle cell trait was 6 0f 8 (75%). The prevalence of malaria parasite in patients with sickle cell trait was lower than in persons without sickle cell trait. It is therefore concluded that the sickle cell trait to a certain degree protects against malaria.

# CHAPTER ONE

## 1.0 Introduction

Forty percent (40%) of the world's population is at risk of contracting malaria. Most of the fatal cases of malaria are in Sub-Saharan Africa, and most are children under the age of five or pregnant women. There are some areas where up to 40% of the children die of malaria. The most effective prevention of malaria in children, as shown in a 1996 World Health Organization study, is protecting them from mosquito bites by having them sleep under bednets dipped in permethrin. In the WHO's pilot study in The Gambia, the death rate among children between birth and 5 years was reduced by 63% by this method.(WHO 2007)

Inside the human host, the malaria parasite first invades the liver cells and then the red blood cells. This Disease is produced when the parasite is inside the red blood cells. When the parasite has matured inside the red blood cells, the cells burst, producing chills and a very high fever. The infected red blood cells and the burst blood cells can cause failure of the liver or the kidneys, hypoglycemia, or cerebral malaria which can include blocking the blood vessels carrying blood to the brain; these events may lead to death. Malaria is an ancient disease; descriptions of its pathology are found in Hippocrates' writings (Beadle et all, 2004). Through the millennium, the distribution of

malaria in the world has changed. It is still a significant health problem in South and Central America as well as in Asia and Africa south of the Sahara. Haemoglobin binds oxygen within red blood cells, which then transport the oxygen to body tissues where it is released from the haemoglobin molecule. The sickle haemoglobin (in a person with a mutant allele) tends to precipitate, or "clump together", within the red blood cell after releasing its oxygen. If the clumping is extensive, the red blood cell assumes an abnormal "sickle" shape. These sickled red blood cells plug the blood vessels, thus preventing normal red blood cell passage and, consequently, depriving the tissue of needed oxygen.

Each person has two copies of the gene that determines whether that person has sickle cell disease. If both copies are "normal" alleles, then only normal hemoglobin is produced. If one of the two alleles is "defective", then that person has a mixture of normal and sickle hemoglobin--a condition known as "sickle cell trait." Sickle cell trait usually results in no ill health effects. If both copies are the "defective" alleles, essentially only sickle hemoglobin is made and the person has sickle cell anemia. Sickle cell anemia is associated with a multitude of medical complications ranging from acute painful crises caused by the plugging of blood vessels to chronic damage to the spleen, kidneys, lungs, heart, muscles and brain. Repeated hospitalization for intravenous pain medication, antibiotic therapy and blood transfusions is undertaken to treat medical problems as they arise. These patients often die early of

overwhelming infection or as a consequence of acute or chronic damage to the body organs. Some progress is being made toward the use of drugs that induce the production of "normal" haemoglobin in sickle cell patients in an effort to decrease the frequency of sickle cell crises. However, bone marrow transplantation, an expensive, high-risk medical procedure, remains the only known cure for this disease.

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

Sickle cell is a serious genetic health disorder that is common in West Africa. Nigeria has the highest prevalence of sickle cell anemia in the world and it has continued to pose significant health challenges among sufferers and health policy makers; occasioned by the morbidity and mortality always associated with the disease. The sickle cell anaemia has consistently posed serious public health concern in sub Saharan Africa, Equatorial Africa, India, Mediterranean and Middle Eastern regions; but the highest prevalence in Sub-Sahara Africa (Oniyangi and Omari, 2006).

Through the millennium, the distribution of malaria in the world has changed

The sickle cell disorder is the commonest genetic disorder in the world with a global birthrate of 300,000; and it originates from a dominant homozygous haemoglobin HbSS (Makani *et al.*, 2010). This abnormal condition is characterized by rigid sickle shape of the red blood cells of individuals with this condition. The abnormal shape and physiology of the red blood cells of children that suffer from this condition reduces the oxygen carrying capacity of the blood cells and leads to symptoms which include shortness of breath, pains, delayed puberty.

## 2.1 MALARIA

Malaria is a parasitic disease which is spread by the female Anopheles mosquitoes. There are about 2 million deaths from malaria each year, making it one of the world's deadliest diseases (Nayyar et al, 2012).

## 2.2 SIGNS AND SYMPTOMS

The signs and symptoms of malaria typically begin 8–25 days following infection however, symptoms may occur later in those who have taken antimalarial medications as prevention (Fairhurst et al, 2010). Initial manifestations of the disease—common to all malaria species—are similar to flu-like symptoms, The classic symptom of malaria is paroxysm—a cyclical occurrence of sudden coldness followed by shivering and then fever and sweating, occurring every two days (tertian fever) in *P. vivax* and *P. ovale* infections, and every three days (quartan fever) for *P. malariae*. *P. falciparum* infection can cause recurrent fever every 36–48 hours or a less pronounced and almost continuous fever.

The terms "sickle cell crisis" or "sickling crisis" may be used to describe several independent acute conditions occurring in patients with sickle cell disease. Sickle cell disease results in anemia and crises that could be of many types including the vaso-occlusive crisis, aplastic crisis, and others. Most episodes of sickle cell crises last between five and seven days. Although infection, dehydration, and acidosis (all of which favor sickling) can act as triggers, in most instances no predisposing cause is identified."

## 2.3 ETIOLOGIC AGENT OF MALARIA

Malaria parasites belong to the genus *Plasmodium* (phylum Apicomplexa). In humans, malaria is caused by *P. falciparum*, *P. malariae*, *P. ovale*, *P. vivax* and *P. knowlesi*. Among those infected, *P. falciparum* is the most common species identified (~75%) followed by *P. vivax* (~20%).Although *P. falciparum* traditionally accounts for the majority of deaths, recent evidence suggests that *P. vivax* malaria is associated with potentially life-threatening conditions about as often as with a diagnosis of *P. falciparum* infection. (Mueller et all, 2007).

## 2.4 TRANSMISSION

The malaria is transmitted from the anopheles mosquito to human through the mosquito bite while the sickle cell trait is transmitted from the parents to the offspring provided either of the parents or both the parents possess an abnormal allele.

## 2.5 RESERVOIR

The reservoir for malaria is the female anopheles mosquito while the reservoir for the sickle cell anaemia is the parents.

## 2.6 TREATMENT

Malaria is treated with antimalarial medications; the ones used depend on the type and severity of the disease. While medications against fever are

commonly used, their effects on outcomes are not clear. Uncomplicated malaria may be treated with oral medications. The most effective treatment for *P. falciparum* infection is the use of artemisinins in combination with other antimalarials

## 2.7 LABORATORY DIAGNOSIS

Owing to the non-specific nature of the presentation of symptoms, diagnosis of malaria in non-endemic areas requires a high degree of suspicion, which might be elicited by any of the following: recent travel history, enlarged spleen, fever, low number of platelets in the blood, and higher-than-normal levels of bilirubin in the blood combined with a normal level of white blood cells. Malaria is usually confirmed by the microscopic examination of blood films or by antigen-based rapid diagnostic tests.

## 2.8 SICKLE CELL ANAEMIA

Sickle cell anaemia is a hereditary blood disorder, characterized by red blood cells that assume an abnormal, rigid, sickle shape. Sickling decreases the cells' flexibility and results in a risk of various life-threatening complications.(Wellems et al,2009)

## 2.9 CAUSES OF SICKLE CELL ANAEMIA

Sickle cell anemia is caused by a "defective" allele (mutant form) of the gene coding for a subunit of the hemoglobin protein.

## 2.10 SICKLE CELL ANAEMIA TREATMENT

Bone marrow transplants are the only known cure for SCD. However, bone marrow transplants are difficult to obtain because of the specific HLA typing necessary. Ideally, a twin family member (syngeneic) or close relative (allogeneic) would donate the bone marrow necessary for transplantation.

## 2.11 LABOURATORY DIAGNOSIS

The full blood count reveals haemoglobin levels with a high reticulocyte count (as the bone marrow compensates for the destruction of sickle cells by producing more red blood cells). In other forms of sickle-cell disease, Haemoglobin levels tend to be higher. A blood film may show features of hyposplenism (target cells and Howell-Jolly bodies). Sickling of the red blood

cells, on a blood film, can be induced by the addition of sodium metabisulfite. The presence of sickle haemoglobin can also be demonstrated with the "sickle solubility test". A mixture of haemoglobin S (Hb S) in a reducing solution (such as sodium dithionite) gives a turbid appearance, whereas normal Hb gives a clear solution.

Abnormal haemoglobin forms can be detected on haemoglobin electrophoresis, a form of gel electrophoresis on which the various types of haemoglobin move at varying speeds. Sickle-cell haemoglobin (HgbS) and haemoglobin C with sickling (HgbSC)—the two most common forms—can be identified from there. The diagnosis can be confirmed with high-performance liquid chromatography (HPLC).

## 2.12 The Sickle Cell - Malaria Interaction.

The allele that causes sickle cell anemia also imparts partial resistance to malaria. In individuals with two "normal" alleles, the malaria parasite can infect the red blood cells. The bursting of these infected cells can cause kidney and liver failure, anemia, hypoglycemia, or block blood vessels to vital organs, such as the brain (causing cerebral malaria). But the red blood cells of individuals with one sickle cell allele are relatively resistant to malaria; furthermore, these individuals do not get sickle cell anemia, if a person lives in an area inhabited by mosquitoes that carry the malaria parasite, then the sickle cell allele can be considered positive in the following sense. One sickle cell allele creates a condition in the blood cells that gives some protection from the malaria parasite, a leading cause of early death in those areas. Sickle-cell is particularly prevalent in areas of high malarial transmission. ( WHO, 2009).

Sickle cell trait is the genetic condition selected for in regions of endemic malaria. Sickle cell disease is a necessary consequence of the existence of the trait condition because of the genetics of reproduction. The precise mechanism by which sickle cell trait imparts resistance to malaria is unknown. A number of factors likely are involved and contribute in varying degrees to the defense against malaria. Red cells from people with sickle trait do not sickle to any significant degree at normal venous oxygen tension. Very low oxygen tensions will cause the cells to sickle, however. For example, extreme exercise

at high altitude increases the number of sickled erythrocytes in venous blood samples from people with sickle cell trait (Martin et al, 2002). Sickle trait red cells infected with the *P. falciparum* parasite deform, presumably because the parasite reduces the oxygen tension within the erythrocytes to very low levels as it carries out its metabolism. Deformation of sickle trait erythrocytes would mark these cells as abnormal and target them for destruction by phagocytes( Luzzatto et al, 2001). Experiments carried out *in vitro* with sickle trait red cells showed that under low oxygen tension, cells infected with *P. falciparum* parasites sickle much more readily than do uninfected cells (Roth Jr et al., 2001). Since sickle cells are removed from the circulation and destroyed in the reticuloendothelial system, selective sickling of infected sickle trait red cells would reduce the parasite burden in people with sickle trait. These people would be more likely to survive acute malarial infections. Other investigations suggest that malaria parasites could be damaged or killed directly in sickle trait red cells. *P. falciparum* parasites cultured in sickle trait red cells died when the cells were incubated at low oxygen tension (Friedman, 1978). In contrast, parasite health and growth were unimpeded in cells maintained at normal atmospheric oxygen tensions. The sickling process that occurs at low oxygen tensions was presumed to harm the parasite in some fashion. Ultrastructural studies showed extensive vacuole formation in *P. falciparum* parasites inhabiting sickle trait red cells that were incubated at low oxygen tension, suggesting metabolic damage to the parasites (Friedman, 2004).

Prolonged states of hypoxia are not physiological, raising questions about degree to which these data can be extrapolated to human beings. However, they do suggest mechanisms by which sickle hemoglobin at the concentrations seen with sickle cell trait red cells could impair parasite proliferation. Other investigations suggest that oxygen radical formation in sickle trait erythrocytes retards growth and even kills the *P. falciparum* parasite (Anastasi, 2003). Sickle trait red cells produce higher levels of the superoxide anion ($O_2$-) and hydrogen peroxide ($H_2O_2$) than do normal erythrocytes. Each compound is toxic to a number of pathogens, including malarial parasites. Homozygous hemoglobin S red cells produce membrane associated hemin secondary to repeated formation of sickle hemoglobin polymers. This membrane-associated hemin can oxidize membrane lipids and proteins (Rank, et al., 2005). Sickle trait red cells normally produce little in the way of such products. If the infected sickle trait red cells form sickle polymer due to the low oxygen tension produced by parasite metabolism, the cells might generate enough hemin to damage the parasites (Orjih, et al., 2005). The immune system is key to weathering attacks by *P. falciparum*. Maternal antibodies passed to newborns prior to birth provide some protection from malaria for the first several months of life. Thereafter, the onus is on the toddler's immune system to provide the needed defense. Epidemiological studies performed in regions with endemic malaria show that antibody titers to *P. falciparum* are lower in children with sickle cell trait than in children with genes only for

hemoglobin A (Cornille-Brogger, et al., 2006). The investigators speculated that lower levels of immune activation might reflect a lower parasite burden in children with sickle cell trait due to clearance of the infected red cells. Analysis of people with sickle cell trait and people homozygous for hemoglobin A in the regions with endemic malaria in fact show a lower mean parasite burden in people with sickle cell trait relative to hemoglobin A homozygotes (Fleming et al, 2004). In contrast, children with sickle cell disease have a high fatality rate, with acute malarial infections being a chief cause of death (Fleming, 2004).Hemoglobin C is also believed to protect against malaria, although data on this point were not conclusive until recently. Hemoglobin C lacks the *in vitro*antimalarial activity of hemoglobin S. Some epidemiological studies found no evidence for protection against malaria in people with either homozygous or heterozygous hemoglobin C (Willcox, et al., 2003). The relatively small number of patients with hemoglobin C in these studies left the conclusions open to question, however. The issue was finally settled in an investigation that included more than 4,000 subjects (Modiano, et al., 2001). Hemoglobin C heterozygotes had significantly fewer episodes of *P. falciparum* malaria than did controls with only hemoglobin A. The risk of malaria was lower still in subjects who were homozygous for hemoglobin C. Homozygous hemoglobin C produces a mild hemolytic anemia and splenomegaly. The much milder phenotype of the condition relative to homozygous hemoglobin S led the investigators to speculate that without

medical intervention for malaria, hemoglobin C would replace hemoglobin S the over the next few thousand years as the dominant "antimalarial" hemoglobin in West Africa. The thalassemias also reached levels of expression in human populations by protecting against malaria. The inbalance in globin chain production characteristic of thalassemia produces membrane oxidation by hemichromes and other molecules that generate reactive oxygen species (Grinberg et al, 2005). Reactive oxygen species also injure and kill malaria parasites (Clark, et al., 2007). *In vitro* malaria toxicity of thalassemic red cells is most easily seen in cells containing hemoglobin H (ß-globin tetramers) (Ifediba et al, 2005). Hemoglobin H occurs most often in people with three-gene deletion alpha-thalassemia (Zhu et al, 2004). The compound heterozygous condition of two-gene deletion alpha thalassemia and hemoglobin Constant Spring also produces erythrocytes that contain hemoglobin H (Derry et al, 2007). Two gene deletion alpha thalassemia also protects the host from malaria, however. The process is difficult to demonstrate with *in vitro* cultures of malaria parasites. Alpha thalassemia may protect against malaria in part by altering the immunue response to parasitized red cells (Luzzi et al, 2002) In any event, epidemiological studies show clear evidence of protection provided by two-gene deletion alpha thalassemia (Flint, et al., 2005). One of the key reasons for the high fatality rate in *P. falciparu* malaria is the occurence of so-called cerebral malaria. Patients become confused, disoriented and often lapse into a terminal coma. Clumps of malaria-

infested red cells adhere to the endothelium and occlude the microcirculation of the brain with deadly consequences. The *P. falciparum* parasite alters the characteristics of the red cell membrane, making them more "sticky". Clusters of parasitized red cells exceed the size of the capillary circulation blocking blood flow and producing cerebral hypoxia. Thalassemic erythrocytes adhere to parasitized red cells much less readily than do their normal counterparts (Carlson, et al., 2006). This alteration would lessen the chance of developing cerebral malaria. The rise to high frequency of alleles that produce red cells deficient in glucose-6-phosphate dehydrogenase activity is one of the most dramatic examples of the selective pressure of malaria on humankind ( Tishkoff, et al., 2001). Reactive oxygen species are formed continually as erythrocytes take up oxygen from the lungs and release it to the preriperal tissues. As noted above, malaria parasites are easily damaged by these reactive oxygen species (Friedman, 2006), Malaria continues to battle back in this struggle, however. The advent of *P. falciparum* parasites that produce their own G-6-PD provides ample evidence of the continuing moves and counter-moves in the battle between man and malaria (Usanga, et al, 2006).

# CHAPTER THREE

## 3.0 METHODOLOGY

### Principles

Sodium metabilsulphite reduces the oxygen tension inducing the typical sickle cell shape of red blood cells.

### Collection of Samples

Fresh blood in any anticoagulant

### Apparatus

Microscope, petri dish, MP kit, slide, cover slip, pipette, electronic weighing balance

### Reagents

Alcohol, assay buffer, 0.2g of sodium metabilsulphite in 10ml (2%) of distilled water, stir until dissolved. Prepare fresh each time,

### Method

The blood samples were taken randomly from selected population. 1 drop of blood is mixed with 0.2g 0f sodium metabilsulphite solution on a microscope slide. It is covered with a cover slip and the edge was sealed with wax or Vaseline mixture. Allow to stand in room temperature for 1 to 4 hours. This was examined

under a microscope with the dry objective for the presence of a sickle form. Thereafter the samples for both patients with sickle cell trait and without sickle cell trait were tested with malaria kit to determine the positive cases.

# CHAPTER FOUR

## 4.0 RESULTS

In positive results the typical sickle cell shaped red blood cell were seen in the samples under microscope. Out of the twenty (20) only eight (8) tested positive for the sickle cell trait.

Negative results were obtained from samples that had no sickle cell trait in the samples when viewed under the microscope.

After the sickling test was done, the malaria test was carried out using the malaria test kit. In positive results from the samples, presence of two colour bands ( one band in the "C" area and another band in the "T"), and in negative results there was just one colour band present.

The total number of samples worked on was twenty (20), eight (8) samples tested positive in the sickling test, 2 of 8 (25%) samples which tested positive in the sickling test had malaria parasite in them.

Twelve (12) samples tested negative in the sickling test, eight (8) samples had malaria parasite among the 12 (66.7%) samples which tested negative in the sickling test. As seen in the table below;

## 4.1 TABLE 1; SAMPLES WITH SICKLING CELL TRAIT

| S.no | Sex of patient | Age of patient | MP test |
|---|---|---|---|
| 1 | F | 25 | N |
| 2 | M | 20 | N |
| 3 | F | 19 | N |
| 4 | M | 25 | N |
| 5 | M | 22 | P |
| 6 | F | 18 | P |
| 7 | M | 20 | N |
| 8 | M | 24 | N |

N;B the letter F represents Female and letter M represents Male

The letter P represents Positive and letter N represents Negative

## 4.2 TABLE 2; SAMPLES WITHOUT SICKLE CELL TRAIT

| S.no | Sex of patient | Age of patient | MP test |
|---|---|---|---|
| 1 | F | 21 | P |
| 2 | F | 18 | N |
| 3 | M | 21 | P |
| 4 | F | 19 | P |
| 5 | M | 22 | P |
| 6 | M | 21 | P |
| 7 | M | 20 | N |
| 8 | M | 21 | N |
| 9 | F | 20 | P |
| 10 | M | 23 | P |
| 11 | M | 21 | P |
| 12 | M | 20 | N |

N;B the letter F represents Female and letter M represents Male

The letter P represents Positive and letter N represents Negative

# CHAPTER FIVE

## 5.0 DISCUSSION

Malaria is a parasitic disease which is spread by the female Anopheles mosquitoes. The signs and symptoms of malaria typically begin 8–25 days following infection however, symptoms may occur later in those who have taken antimalarial medications as prevention (Fairhurst et al,. 2010). Sickle cell anaemia is a hereditary blood disorder, characterized by red blood cells that assume an abnormal, rigid, sickle shape. The allele that causes sickle cell anemia also imparts partial resistance to malaria. In individuals with two "normal" alleles, the malaria parasite can infect the red blood cells. The red blood cells of individuals with one sickle cell allele are relatively resistant to malaria (Wellems et al,.2009)

This work is different from other results because the prevalence of malaria parasite among patients with sickle cell trait in Ekiti state is lower. From these results it can be inferred that the sickle cell trait protects against malaria because those that had the trait had lower frequency of malaria parasites.

## 5.1 CONCLUSION AND RECOMMENDATION

A total no of 20 samples of person with and without sickle cell trait from the hospitals and clinics were tested accordingly, the prevalence of the malaria parasite among patients with sickle cell trait was 2 of 8 (25%), and that of sickle cell trait patients without malaria is 6 0f 8 (75%). The prevalence of malaria parasite in patients with sickle cell trait was lower than in persons without sickle cell trait. It is therefore concluded that the sickle cell trait to a certain degree protects against malaria.

It is recommended that persons without sickle cell trait should take malaria treatment from time to time so as to keep them from malaria parasite infection since persons with sickle cell trait in them have partial resistance to malaria parasite infection.

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