**RELATIONSHIP BETWEEN ANAEMIA, GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY AND OXIDATIVE STRESS AMONG MALARIA PATIENTS VISITING ESUT TEACHING HOSPTIAL ENUGU NIGERIA.**

**BY**

**NWABUEZE CHINENYE.N.**

**U15/NAS/BCH/035**

**SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCES (B.Sc.) DEGREE IN BIOCHEMISTRY**

**DEPARTMENT OF CHEMICAL SCIENCES**

**FACULTY OF NATURAL AND APPLIED SCIENCES**

**GODFREY OKOYE UNIVERSITY, UGWUOMU-NIKE**

**ENUGU, ENUGU STATE**

**JULY, 2018.**

**TITLE PAGE**

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**ENUGU, ENUGU STATE**

**SUPERVISOR:**

**MR. ENGWA AZEH GODWILL**

**DATE: JULY, 2018.**

**CERTIFICATION**

I Nwabueze chinenye Nnaji an undergraduate student of the Department of Chemical Sciences with registration number U15/NAS/BCH , hereby certify that the work embodied in the project is original and has not been submitted in part or full in any other Degree programme of this University.

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# APPROVAL

This research titled “Relationship between anamia,oxidative stress and Glucose-6-phosphate dehydrogenase deficiency among malaria in patient visting ESUTH teaching hospital Enugu Nigeria.” has been assessed and approved by the Department of Chemical Sciences and Faculty of Natural and Applied Sciences.

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 Name of Supervisor Signature Date

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 Name of HOD, Signature Date

 Department of Biochemistry

# DEDICATION

I dedicate this work to Almighty God and for his intimate mercy upon me throughout this research work.

**ACKNOWLEDGEMENT**

I give thanks to Almighty God for his abundant grace and blessings upon my life.

I sincerely acknowledge my supervisor Mr. Engwa Azeh Godwill, my H.O.D Chemical Science Department, Mr. Ayuk E.L, and Mr. Ugwu Francis for their full support and listening ears to me always.

To all my lecturers I will ever remain grateful. I say a big thank you for having led me thus far in my academic pursuits. To all the Staff of the Department of Chemical Sciences, may God reward you.

I greatly appreciate my husband and my parents for their endless support, morally, financially, and spiritually.

Also, I say a big thank you to my mates who supported me during my project.

**ABSTRACT**

Malaria is a significant public health problem in the world especially in in sub-Saharan Africa. One of the key contributory factors to the development and progression of malaria and its complications is oxidative stress, a condition characterized by increased production of free radicals or impaired antioxidant defence system. Glucose-6-phosphate dehydrogenase (G-6-PD) produces NADPH which intends regenerate reduced glutathione (GSH), an antioxidant which helps in the removal of free radicals thereby preventing oxidative stress, Hence, this study was aimed to investigate the relationship between G-6-PD, oxidative stress and malaria infection in patients visiting ESUT Teaching Hospital Parklane, Enugu. The recruited patients following their informed consent were screened for malaria by RDT and Microscopy methods and their baseline parameters including age, gender, etc. were obtained using a questionnaire. Whole blood was collected and used for the determination of malaria infection, oxidative stress, lipid peroxidation and protein oxidation, anaemia and as well as the G-6-PD status in patients. A total of 101 patients were recruited including 30 male and 71 female among which 86 had malaria positive while 15 tested malaria negative. Comparing RDT and Microscopy techniques in diagnosing Malaria, showed RDT to have a low performance in in diagnosing malaria using microscopy as standardwith a sensitivity of 10.47% and of accuracy 23.76%. All the baseline characteristics of study participants was not significantly different (p = 0.946) among the malaria and non- malaria patients. Among the G6PD deficient patients, 17.9% were found to be anaemic while 13.1% were non-anaemic whereas among the non-deficient patients, 39.3% were anaemic while 29.8% were non-anaemic. As such, there was no significant relationship (*p* = 0.946) between G6PD deficiency and among the malaria patients. Comparison of anaemia and oxidative stress indices among malaria patients showed significantly (*p*<0.05) low level of haemoglobin and haematocrit concentrations, but there no significant difference (p>0.05) of MDA and protein oxidation level between anemic and non-anaemic patients with malaria. Interaction between anaemia and G6PD deficiency on study parameters, showed no significant (*p*<0.05) relationship on haemoglobin, haematocrit, MDA and protein oxidation level in malaria patients. In conclusion, this study showed the no association between anaemia, oxidative stress and G-6-PD deficiency among malaria patients. Further studies are needed to ascertain these findings as oxidative stress is implicated in the pathogenesis of malaria.

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**CHAPTER ONE**

**1.0 INTRODUCTION**

**1.1 Background of study**

Malaria is a mosquito-borne infectious disease affecting humans and other animals caused by parasitic protozoans (a group of single-celled microorganisms) belonging to the *Plasmodium* type (WHO, 2014). According to the World Health Organization (WHO), malaria is a significant public health problem in more than 100 countries and causes an estimated 200 million infections each year, with more than 500 thousand deaths annually. Over 90% of these deaths occur in sub-Saharan Africa, where the disease is estimated to kill one child every 30 seconds (WHO, 2011). In other areas of the world, malaria causes substantial morbidity, especially in the rural areas of some countries in Asia and South America. Malaria causes symptoms that typically include fever, tiredness, vomiting, and headaches. In severe cases it can cause yellow skin, seizures, coma, or death (Caraballo, 2014). Symptoms usually begin ten to fifteen days after being bitten, If not properly treated, people may have recurrences of the disease months later. The disease is most commonly transmitted by an infected female Anopheles mosquito. The mosquito bite introduces the parasites from the mosquito's saliva into a person's blood (WHO, 2014). The parasites travel to the liver where they mature and reproduce. Five species of *Plasmodium* can infect and be spread by humans. (Caraballo, 2014). Most deaths are caused by *Plasmodium falciparum.*

 The role of oxidative stress during malaria infection is still unclear. Some authors suggest a protective role, whereas others claim a relation to the physiopathology of the disease (Sohail *et al.,* 2007). However, recent studies suggest that the generation of reactive oxygen and nitrogen species (ROS and RNS) associated with oxidative stress, plays a crucial role in the development of systemic complications caused by malaria. Malaria infection induces the generation of hydroxyl radicals (OH•) in the liver, which most probably is the main reason for the induction of oxidative stress and apoptosis (Guha *et al.,* 2006). Additionally, Atamna et al. (1993) observed that erythrocytes infected with P. falciparum produced OH• radicals and H2O2 about twice as much compared to normal erythrocytes. Higher level of this free radicals can lead to oxidative stress.

Oxidative stress, termed as an imbalance between production and elimination of reactive oxygen species (ROS) leading to plural oxidative modifications of basic and regulatory processes, can be caused in different ways. Increased steady-state ROS levels can be promoted by drug metabolism, over-expression of ROS-producing enzymes, or ionizing radiation, as well as due to deficiency of antioxidant enzymes. The consequence of oxidative stress once it is high, it can cause damage to the brain, metabolic disorders affecting electron transport chain. Reactive oxygen species (ROS), generated by endogenous and exogenous sources, cause significant damage to macromolecules, including DNA (Salmon *et al.,* 2004).

Furthermore, Spermatozoa are highly vulnerable to oxidative attack because they lack significant antioxidant protection due to the limited volume and restricted distribution of cytoplasmic space in which to house an appropriate armoury of defensive enzymes. In particular, sperm membrane lipids are susceptible to oxidative stress because they abound in significant amounts of polyunsaturated fatty acids. Susceptibility to oxidative attack is further exacerbated by the fact that these cells actively generate reactive oxygen species (ROS) in order to drive the increase in tyrosine phosphorylation associated with sperm capacitation. However, this positive role for ROS is reversed when spermatozoa are stressed. Under these conditions, they default to an intrinsic apoptotic pathway characterised by mitochondrial ROS generation, loss of mitochondrial membrane potential, caspase activation, phosphatidylserine exposure and oxidative DNA damage. In responding to oxidative stress, spermatozoa only possess the first enzyme in the base excision repair pathway, 8-oxoguanine DNA glycosylase. This enzyme catalyses the formation of abasic sites, thereby destabilising the DNA backbone and generating strand breaks. Because oxidative damage to sperm DNA is associated with both miscarriage and developmental abnormalities in the offspring, strategies for the amelioration of such stress, including the development of effective antioxidant formulations, are becoming increasingly urgent (Aitken *et at.,* 2016).

The process of lipid peroxidation involves a complex chain reaction utilizing the interaction of oxygen-derived species with polyunsaturated fatty acids (e.g. docosahexaenoic acid, linoleic acid and arachidonic acid), resulting in highly reactive electrophilic aldehydes and free radicals (Esterbauer *et al*., 1991). This process is extremely detrimental to cellular functions as it disrupts membrane integrity, fluidity and function (Esterbauer *et al.,* 1991). Lipid peroxidation is a self-propagating process involving initiation and propagation steps which continue through an ongoing free radical chain reaction until termination occurs. The retina is particularly prone to lipid peroxidation since it is highly enriched in polyunsaturated fatty acids (PUFAs) (Catalase). The predominant PUFA in photoreceptor outer segments is docosahexanoic acid which is the most unsaturated fatty acid in the body. Lifelong accumulation of chronic oxidative damage will lead to dysfunction in retinal cells and increase their susceptibility to exogenous and endogenous insults eventually culminating in loss of visual function and cell death (Esterbauer *et al*.,1991). Malaria infection has been found to be associated with lipid peroxidation accompanying reduction in antioxidant capacity of the infected patients especially *Plasmodium falciparum infection*. Instantaneous reduction in antioxidant potency in tandem with increased lipid peroxidation is also observed to be equally accountable for development of oxidative stress in malaria patients ([Das and Nanda, 1999](https://scialert.net/fulltextmobile/?doi=ajbs.2011.506.513#549164_ja); [Upadhyay *et al*., 2011](https://scialert.net/fulltextmobile/?doi=ajbs.2011.506.513#763311_ja); [Egwunyenga *et al*., 2004](https://scialert.net/fulltextmobile/?doi=ajbs.2011.506.513#763202_ja)). Any infection, including malaria, activates the immune system of body thereby causing release **of** [**reactive oxygen species**](http://www.scialert.net/asci/result.php?searchin=Keywords&cat=&ascicat=ALL&Submit=Search&keyword=reactive+oxygen+species) as an antimicrobial action ([Kulkarni *et al*., 2003](https://scialert.net/fulltextmobile/?doi=ajbs.2011.506.513#82588_ja)). In addition to host’s immune system, malaria parasite also stimulates certain cells in production of [**reactive oxygen species**](http://www.scialert.net/asci/result.php?searchin=Keywords&cat=&ascicat=ALL&Submit=Search&keyword=reactive+oxygen+species) thereby resulting in hemoglobin degradation ([Loria *et al*., 1999](https://scialert.net/fulltextmobile/?doi=ajbs.2011.506.513#549198_ja); [Pradines *et al*., 2005](https://scialert.net/fulltextmobile/?doi=ajbs.2011.506.513#115878_ja)). One of the major reasons for development of malarial anemia seems to be oxidative stress ([Das and Nanda, 1999](https://scialert.net/fulltextmobile/?doi=ajbs.2011.506.513#549164_ja); [Kremsner *et al*., 2000](https://scialert.net/fulltextmobile/?doi=ajbs.2011.506.513#82587_ja)) while changes in micronutrient metabolism alter disease progression and severity ([Singotamu *et al*., 2006](https://scialert.net/fulltextmobile/?doi=ajbs.2011.506.513#123061_ja)).

Proteins are the largest constituent of the cellular milieu and are frequent targets of oxidative damage (Stadtman, 2004). Protein oxidation can involve direct reaction with amino acids, cleavage of the polypeptide chain, and conversion of the protein to derivatives that are highly sensitive to proteolytic degradation. It has also been established that all of these protein modifications can be mediated by metal-catalyzed oxidation systems. All amino acid residues of proteins are potential targets for oxidation by HO· or by H2O2 in the presence of metal ions. For example, oxidation of tyrosine residues is damaging to the red blood cells, as this amino acid is converted to a 3,4-dihydroxyphenylanine derivative, which itself can undergo redox cycling to generate further ROS (Sugiura and Ichinose, 2011).

Antioxidants are molecules that inhibit or quench free radical reactions and delay or inhibit cellular damage (Young *et al*., 2001). Though the antioxidant defenses are different from species to species, the presence of the antioxidant defense is universal. Antioxidants exists both in enzymatic and non-enzymatic forms in the intracellular and extracellular environment. . Enzymatic antioxidants work by breaking down and removing free radicals. The antioxidant enzymes convert dangerous oxidative products to hydrogen peroxide (H2O2) and then to water, in a multi-step process in presence of cofactors such as copper, zinc, manganese, and iron. Non-enzymatic antioxidants work by interrupting free radical chain reactions (Young *et al*.,2001).

The antioxidants can also be categorized according to their size, the small-molecule antioxidants and large-molecule antioxidants. The small-molecule antioxidants neutralize the ROS in a process called radical scavenging and carry them away.

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common enzymopathological disease in humans. This disease is described as a widespread, heritable, X-chromosome linked abnormality (Reclose *et al*., 2000). It is estimated that it affects approximately 400 million people worldwide (Daloii *et al.,* 2004). This disease is seen most frequently in approximately all of Africa, Asia, and the countries near the mediterranean Sea (Frank, 2005). G6PD enzyme was demonstrated to play an active role in survival of erythrocytes. It is known that in the pentose phosphate pathway of erythrocytes, glucose-6 phosphate dehydrogenase (G6PD) enzyme provides the production of NADPH and Glutathione (**GSH**). GSH, produced by pentose phosphate pathway can react with H2O2 and reduce it to H2O. This prevents the generation of oxidative stress within red blood cells; oxidative stress can be induced in erythrocytes whose G6PD enzymes are deficient. In this situation, GSH is not produced and H2O2 is not reduced to H2O, leading to oxidative stress and hemolysis.

**1.2 Justification / Need for study**

The need for this study is to carefully ascertain whether G6pD deficiency has an impact on oxidative stress and malaria infection. To known if G6pD can promote malaria infection in patient. This study is equally designed to give information about the prevalence of G6PD and malaria infection among malaria patient in Enugu metropolis.

**1.3 Statement of the problem**

So many reasons have been attributed to susceptibility of G-6-PD deficient patients to malaria infection. Recall that the pentose phosphate pathway is essential in producing enough NADPH capable of reducing oxidized glutathione but when there is a default in the production of enough NADPH from PPP as the case may be in G-6-PD deficient patients, leading to a decrease in the level of reduced glutathione which leads to an increased in oxidative stress – a possible risk factor in the development of malaria infection will be complicated.

1.**4 Aim of the study**

This study was undertaken to evaluate the relationship between oxidative stress among malaria patients visiting Enugu State University Teaching Hospital, Parklane, Enugu Nigeria.

**1.5 Objective of the study**

1. To screen patients for malaria infection.

2. To assess anaemia in malaria patients.

3. To assess G6PD deficiency in malaria patients.

4. To assess oxidative stress indices (lipid peroxidation and protein oxidation) in malaria patients.

**1.6 Limitations of the study**

Recruitment of participants into the study.

**CHAPTER TWO**

**2.0 Literature Review**

**2.1 Malaria**

 Malaria is a common and life-threatening disease in many tropical and subtropical areas.It is also mosquito-borne infectious disease affecting humans and other animals caused by parasitic protozoans (a group of single-celled microorganisms) belonging to the Plasmodium type (WHO, 2014). According to the World Health Organization (WHO), malaria is a significant public health problem in more than 100 countries and causes an estimated 200 million infections each year, with more than 500 thousand deaths annually. Over 90% of these deaths occur in sub-Saharan Africa, where the disease is estimated to kill one child every 30 seconds (WHO, 2011). In other areas of the world, malaria causes substantial morbidity, especially in the rural areas of some countries in Asia and South America (Caraballo, 2004).

**2.1.1 Taxonomic Classifications of plasmodium**

|  |  |
| --- | --- |
| Kingdom Subkingdom Phylum Class Order Family Genus Species | Protista Protozoa Apicomplexa Sporozoasida Eucoccidiorida Plasmodiidae *Plasmodium* *falciparum,  malariae,  ovale,  vivax* |

*Plasmodium* belongs to the [phylum](https://en.wikipedia.org/wiki/Phylum) [Apicomplexa](https://en.wikipedia.org/wiki/Apicomplexa), a taxonomic group of single-celled parasites with characteristic [secretory organelles](https://en.wikipedia.org/wiki/Apicomplexa#General_features) at one end of the cell (Morrison, 2009). Within Apicomplexa, *Plasmodium* is within the [order](https://en.wikipedia.org/wiki/Order_%28biology%29) [Haemosporida](https://en.wikipedia.org/wiki/Haemosporida), a group that includes all apicomplexans that live within blood cells. Votypka (1885) Based on the presence of the pigment [hemozoin](https://en.wikipedia.org/wiki/Hemozoin) and the method of [asexual reproduction](https://en.wikipedia.org/wiki/Merogony), the order is further split into four families, of which *Plasmodium* is in the [family](https://en.wikipedia.org/wiki/Family_%28biology%29) [Plasmodiidae](https://en.wikipedia.org/wiki/Plasmodiidae).

The genus *Plasmodium* consists of over 200 species, generally described on the basis of their appearance in blood smears of infected vertebrates. These species have been categorized on the basis of their morphology and host range into 14 subgenera as described below (Perkins, 2015).

* Subgenus [*Asiamoeba*](https://en.wikipedia.org/wiki/Asiamoeba) (Telford, 1988) – reptiles
* Subgenus [*Bennettinia*](https://en.wikipedia.org/wiki/Bennettinia) (Valkiunas, 1997) – birds
* Subgenus [*Carinamoeba*](https://en.wikipedia.org/wiki/Carinamoeba) (Garnham, 1966) – reptiles
* Subgenus [*Giovannolaia*](https://en.wikipedia.org/wiki/Giovannolaia) (Corradetti, *et al*. 1963) – birds
* Subgenus [*Haemamoeba*](https://en.wikipedia.org/wiki/Haemamoeba) (Corradetti, *et al*. 1963) – birds
* Subgenus [*Huffia*](https://en.wikipedia.org/wiki/Huffia) (Corradetti, *et al*. 1963) – birds
* Subgenus [*Lacertamoeba*](https://en.wikipedia.org/wiki/Lacertamoeba) (Telford, 1988) – reptiles
* Subgenus [*Laverania*](https://en.wikipedia.org/wiki/Laverania) (Bray, 1958) – great apes, humans
* Subgenus [*Novyella*](https://en.wikipedia.org/wiki/Novyella) (Corradetti, *et al*. 1963) – birds
* Subgenus [*Ophidiella*](https://en.wikipedia.org/wiki/Ophidiella) (Telford, 1988) – reptiles
* Subgenus [*Paraplasmodium*](https://en.wikipedia.org/wiki/Paraplasmodium) (Telford, 1988) – reptiles
* Subgenus *Plasmodium* (Bray, 1955) – monkeys and apes
* Subgenus [*Sauramoeba*](https://en.wikipedia.org/wiki/Sauramoeba) (Garnham, 1966) – reptiles
* Subgenus [*Vinckeia*](https://en.wikipedia.org/wiki/Vinckeia) (Garnham, 1964) – lower mammals inc. lower primates

Species infecting [monkeys](https://en.wikipedia.org/wiki/Monkey) and [apes](https://en.wikipedia.org/wiki/Apes) (the higher [primates](https://en.wikipedia.org/wiki/Primates)) with the exceptions of *P. falciparum* and *P. reichenowi* (which together make up the subgenus *Laverania*) are classified in the subgenus *Plasmodium*. Parasites infecting other [mammals](https://en.wikipedia.org/wiki/Mammal) including lower primates ([lemurs](https://en.wikipedia.org/wiki/Lemur) and others) are classified in the subgenus *Vinckeia*. The five subgenera *Bennettinia*, *Giovannolaia*, *Haemamoeba*, *Huffia*, and *Novyella* contain the known avian malarial species (Valkiunas, 2004). The remaining subgenera: *Asiamoeba*, *Carinamoeba*, *Lacertamoeba*, *Ophidiella*, *Paraplasmodium*, and *Sauramoeba* contain the diverse groups of parasites found to infect reptiles (Telford S 1988).

**2.1.2 Epidemiology of Malaria**

Malaria infection is still to be considered a major public health problem in those 106 countries where the risk of contracting the infection with one or more of the Plasmodium species exists. According to estimates from the World Health Organization, over 200 million cases and about 655.000 deaths have occurred in 2010. Estimating the real health and social burden of the disease is a difficult task, because many of the malaria endemic countries have limited diagnostic resources, especially in rural settings where conditions with similar clinical picture may coexist in the same geographical areas. Moreover, asymptomatic parasitaemia may occur in high transmission areas after childhood, when anti-malaria semi-immunity occurs. Malaria endemicity and control activities are very complex issues, that are influenced by factors related to the host, to the parasite, to the vector, to the environment and to the health system capacity to fully implement available anti-malaria weapons such as rapid diagnostic tests, artemisinin-based combination treatment, impregnated bed-nets and insecticide residual spraying while waiting for an effective vaccine to be made available (Beatrice *et al.,* 2012).



**Fig. A: Microscopic View of Malaria Parasite**



**Fig. B: Microscopic Nature of Different Malaria Plasmodia**

**2.1.3 Causes of Maleria**

Malaria is caused by the protozoan parasite *Plasmodium*. When a bite from the female *Anopheles* mosquito infects the body with *Plasmodium*. Only the *Anopheles* mosquito can transmit malaria.

The successful development of the parasite within the mosquito depends on several factors, the most important being humidity and ambient temperatures.

When an infected mosquito bites a human host, the parasite enters the bloodstream and lays dormant within the liver.

The host will have no symptoms for an average of [10.5 days](https://malariajournal.biomedcentral.com/articles/10.1186/1475-2875-13-500), but the malaria parasite will begin to multiply during this time.

The new malaria parasites are then released back into the bloodstream, where they infect red blood cells and multiply further. Some malaria parasites remain in the liver and are not released until later, resulting in recurrence.

An unaffected mosquito becomes infected once it feeds on an infected individual. This restarts the cycle.

**2.1.4** **Nature of the disease**

Malaria is an acute febrile illness with an incubation period of 7 days or longer. Thus, a febrile illness developing less than 1 week after the first possible exposure is not malaria. The most severe form is caused by *P. falciparum*; variable clinical features include fever, chills, headache, muscular aching and weakness, vomiting, cough, diarrhea and abdominal pain. Other symptoms related to organ failure may supervene, such as acute renal failure, pulmonary edema, generalized convulsions, circulatory collapse, followed by coma and death. The initial symptoms, which may be mild, may not be easy to recognize as being due to malaria. It is important that the possibility of *falciparum* malaria is considered in all cases of unexplained fever starting at any time between 7 days after the first possible exposure to malaria and 3 months (or, rarely, later) after the last possible exposure. Any individual who experiences a fever in this interval should immediately seek diagnosis and effective treatment, and inform medical personnel of the possible exposure to malaria infection. *Falciparum* malaria may be fatal if treatment is delayed beyond 24 hours after the onset of clinical symptoms. Young children, pregnant women, people who are immunosuppressed and elderly travelers are particularly at risk of severe disease. Malaria, particularly *P. falciparum*, in non-immune pregnant travelers increases the risk of maternal death, miscarriage, stillbirth and neonatal death. Human malaria caused by other *Plasmodium* species results in significant morbidity but is rarely life-threatening. Cases of severe *P. vivax* malaria have been reported among populations living in (sub) tropical countries with malaria risk. *P. vivax* and *P. ovale* can remain dormant in the liver; relapses caused by these persistent liver forms (“hypnozoites”) may appear months − and, rarely, several years − after exposure. Relapses are not prevented by current chemoprophylactic regimens, with the exception of primaquine. Latent blood infection with *P. malariae* may be present for many years, but it is very rarely life-threatening (WHO, 2017).

**2.1.5 Pathogenesis of Malaria Parasite**

About 3.3 billion people – half the world's population – are at risk of malaria due to the main causative parasite, Plasmodium falciparum (WHO, 2008). Over 200 million cases of malaria occur each year, 90% of which occur in Africa, and 655 000 people died from the disease in 2010, making it one of the world's most important health problems. Increasing resistance of the parasite to currently available drugs has created an urgent need to discover new treatments (Crawley *et al.,* 2010). However, this requires an improved understanding of the pathogenesis of malaria.

Malarial infection begins when a person is bitten by an infected female anopheles mosquito and Plasmodium spp (species) parasites in the form of sporozoites are injected into the bloodstream. The sporozoites travel to the liver, multiplying asexually over the next 7–10 days. During this time there are no symptoms. The parasites, now in the form of merozoites, emerge from the liver cells in vesicles and travel through the heart to the capillaries of the lungs. The vesicles eventually disintegrate, releasing the merozoites to enter the bloodstream where they invade and multiply in erythrocytes. When the cells burst, the parasites invade more erythrocytes. Clinical symptoms, including fever, occur in synchrony with the rupture of infected erythrocytes and the release of erythrocyte and parasite debris, including malarial pigment (hemozoin) and glycophosphatidylinositol, the putative ‘malaria toxin’ (Schofield *et al.,* 1993, Clark *et al.,* 2003). In some infected blood cells, instead of replicating asexually, the merozoites develop into sexual forms (gametocytes), which circulate in the bloodstream and are ingested during mosquito bites. The ingested gametocytes develop in the mosquito into mature sex cells (gametes) which develop into ookinetes that actively burrow through the mid-gut wall of the mosquito and form oocysts, in which develop thousands of active sporozoites. The oocyst eventually bursts, releasing sporozoites that travel to the salivary glands of the mosquito. The cycle of human infection begins again when the mosquito bites another person (Perkins *et al.,* 2011).



**Fig. C: Life Cycle of *Plasmodium* in Mosquito**



**Fig D. Life Cycle of Malaria Parasite**

**2.1.6 Signs and Symptoms of Malaria**

The associated symptoms of malaria include severe anemia, fever, thrombocytopenia, chills, headache, vomiting, muscle ache, anorexia, rigor, diarrhea, abdominal discomfort, cough, seizures, respiratory distress, hypoglycemia, metabolic acidosis, hyperlactemia, coma associated with increased intracranial pressure (cerebral malaria), retinopathy, and complications of pregnancy, including preterm birth and low birth weight due to fetal growth restriction (FGR). As noted, the symptoms are associated with the rupture of the infected erythrocytes and the release of putative malaria toxins, which activate peripheral blood mononuclear cells and stimulate the release of cytokines. It is believed that the balance between pro-inflammatory and anti-inflammatory cytokines, chemokines, growth factors, and effector molecules determines disease severity (Langhorne *et al.,* 2008). Studies have reported increased IL-1B, IL-6, IL-8, and TNF-alpha in late-onset severe disease, and a low IL-10:TNF-alpha ratio. The role of cytokines, however, remains contradictory and unclear (Perkins *et al.,* 2011). Alterations in retinoids (vitamin A and its congeners) also occur in malaria, but the precise role of retinoids in the disease may be different and even opposite to the traditional focus on vitamin A deficiency and supplementation. This paper presents a new theory on the pathogenesis of malaria, suggesting that an endogenous form of hypervitaminosis A induced by the parasite contributes significantly to the signs and symptoms of the disease.

Malaria symptoms can be [classified](https://www.cdc.gov/malaria/about/disease.html) into two categories: uncomplicated and severe malaria.

**a) Uncomplicated Malaria:**

This is diagnosed when symptoms are present, but there are no signs to indicate severe infection or dysfunction of the vital organs.This form can become severe malaria if left untreated, or if the host has poor or no immunity.Symptoms of uncomplicated malaria typically last 6 to 10 hours and recur every second day. Some strains of the parasite can have a longer cycle or cause mixed symptoms.As symptoms resemble those of flu, they may be undiagnosed or misdiagnosed in areas where malaria is less common.

In uncomplicated malaria, symptoms progress as follows, through cold, hot, and sweating stages:

* A sensation of cold with shivering
* [Fever](https://www.medicalnewstoday.com/articles/168266.php), [headaches](https://www.medicalnewstoday.com/articles/73936.php), and vomiting
* Seizures sometimes occur in younger people with the disease
* Sweats, followed by a return to normal temperature, with [tiredness](https://www.medicalnewstoday.com/articles/248002.php)

In areas where malaria is common, many patients recognize the symptoms as malaria and treat themselves without visiting a doctor.

**b). Severe Malaria**

In severe malaria, clinical or laboratory evidence shows signs of vital organ dysfunction.

Symptoms of severe malaria include:

* Fever and chills
* Impaired consciousness
* Prostration, or adopting a prone position
* Multiple convulsions
* Deep breathing and respiratory distress
* Abnormal bleeding and signs of [anemia](https://www.medicalnewstoday.com/articles/158800.php)
* Clinical [jaundice](https://www.medicalnewstoday.com/articles/165749.php) and evidence of vital organ dysfunction .Severe malaria can be fatal without treatment.

**2.1.7 Diagnosis of malaria**

Malaria diagnosis involves identifying malaria parasites or antigens/products in patient blood. Although this may seem simple, the diagnostic efficacy is subject to many factors. The different forms of the 5 malaria species; the different stages of erythrocytic schizogony, the endemicity of different species, the interrelation between levels of transmission, population movement, parasitemia, immunity, and signs and symptoms; drug resistance, the problems of recurrent malaria, persisting viable or non-viable parasitemia, and sequestration of the parasites in the deeper tissues, and the use of chemoprophylaxis or even presumptive treatment on the basis of clinical diagnosis, can all influence the identification and interpretation of malaria parasitemia in a diagnostic test (Bell *et al*.,2005,Reyburn *et al*.,2007).

Different methods of malaria diagnosis include;

**A). Microscopy:**

The most economic, preferred, and reliable diagnosis of malaria is microscopic examination of [blood films](https://en.wikipedia.org/wiki/Blood_film) because each of the four major parasite species has distinguishing characteristics. Two sorts of blood film are traditionally used. Thin films are similar to usual blood films and allow species identification because the parasite's appearance is best preserved in this preparation. Thick films allow the microscopist to screen a larger volume of blood and are about eleven times more sensitive than the thin film, so picking up low levels of infection is easier on the thick film, but the appearance of the parasite is much more distorted and therefore distinguishing between the different species can be much more difficult. With the pros and cons of both thick and thin smears taken into consideration, it is imperative to utilize both smears while attempting to make a definitive diagnosis (Warhurst et al., 1996).

From the thick film, an experienced microscopist can detect parasite levels (or [parasitemia](https://en.wikipedia.org/wiki/Parasitemia)) as few as 5 parasites/[µL](https://en.wikipedia.org/wiki/Litre) blood (Richard et al., 2006). Diagnosis of species can be difficult because the early trophozoites ("ring form") of all four species look similar and it is never possible to diagnose species on the basis of a single ring form; species identification is always based on several trophozoites.A new system, by [www.foldscope.com](http://www.foldscope.com) provides a $1 paper microscope and centrifuge that can be deployed to rural areas in the third world.

*Plasmodium malariae* and *P. knowlesi* (which is the most common cause of malaria in [South-east Asia](https://en.wikipedia.org/wiki/South-east_Asia)) look very similar under the microscope. However, *P. knowlesi* parasitemia increases very fast and causes more severe disease than *P. malariae*, so it is important to identify and treat infections quickly. Therefore, modern methods such as PCR (see "Molecular methods" below) or [monoclonal antibody](https://en.wikipedia.org/wiki/Monoclonal_antibody) panels that can distinguish between the two should be used in this part of the world (McCutchan et al., 2008).

**B). Rapid Diagnostic Test:**

For areas where microscopy is not available, or where laboratory staff are not experienced at malaria diagnosis, there are commercial antigen detection tests that require only a drop of blood (Pattanasin et al., 2003). Immunochromatographic tests (also called: [Malaria Rapid Diagnostic Tests](https://en.wikipedia.org/wiki/Malaria_antigen_detection_tests), Antigen-Capture Assay or "[Dipsticks](https://en.wikipedia.org/wiki/Dipsticks)") have been developed, distributed and fieldtested. These tests use finger-stick or venous blood, the completed test takes a total of 15–20 minutes, and the results are read visually as the presence or absence of colored stripes on the dipstick, so they are suitable for use in the field. The threshold of detection by these rapid diagnostic tests is in the range of 100 parasites/µl of blood (commercial kits can range from about 0.002% to 0.1% parasitemia) compared to 5 by thick film microscopy. One disadvantage is that dipstick tests are qualitative but not quantitative – they can determine if parasites are present in the blood, but not how many.

The first rapid diagnostic tests were using *Plasmodium* [glutamate dehydrogenase](https://en.wikipedia.org/wiki/Glutamate_dehydrogenase) as antigen (Ling et al., 1986). PGluDH was soon replaced by *Plasmodium* [lactate dehydrogenase](https://en.wikipedia.org/wiki/Lactate_dehydrogenase) (pLDH). Depending on which [monoclonal antibodies](https://en.wikipedia.org/wiki/Monoclonal_antibodies) are used, this type of assay can distinguish between different species of human malaria parasites, because of antigenic differences between their pLDH isoenzymes. Antibody tests can also be directed against other malarial antigens such as the *P. falciparum* specific HPR2.

Modern rapid diagnostic tests for malaria often include a combination of two antigens such as a *P. falciparum*. specific antigen e.g. histidine-rich protein II (HRP II) and either a *P. vivax* specific antigen e.g. *P. vivax* LDH or an antigen sensitive to all *plasmodium* species which affect humans e.g. pLDH. It should be noted that such tests do not have a [sensitivity](https://en.wikipedia.org/wiki/Sensitivity_and_specificity#Sensitivity) of 100% and where possible, microscopic examination of blood films should also be performed.

**C). Molecular Methods**:

Molecular methods are available in some clinical laboratories and rapid real-time assays (for example, [QT-NASBA](https://en.wikipedia.org/wiki/Real-time_polymerase_chain_reaction) based on the [polymerase chain reaction](https://en.wikipedia.org/wiki/Polymerase_chain_reaction)) are being developed with the hope of being able to deploy them in endemic areas (Mens et al., 2006).

PCR (and other molecular methods) is more accurate than microscopy. However, it is expensive, and requires a specialized laboratory. Moreover, levels of parasitemia are not necessarily correlative with the progression of disease, particularly when the parasite is able to adhere to blood vessel walls. Therefore, more sensitive, low-tech diagnosis tools need to be developed in order to detect low levels of parasitemia in the field (Redd et al., 2006).

Another approach is to detect the [iron crystal byproduct of hemoglobin](https://en.wikipedia.org/wiki/Hemozoin) that is found in malaria parasites feasting on red blood cells, but not found in normal blood cells. It can be faster, simpler and precise than any other method. Researchers at [Rice University](https://en.wikipedia.org/wiki/Rice_University) have published a preclinical study of their new tech that can detect even a single malaria-infected cell among a million normal cells,. They claim it can be operated by nonmedical personal, produce zero false-positive readings, and it doesn’t need a needle or any damage done (news.rice.edu).

## **2.1.8 Treatment**

Treatment aims to eliminate the *Plasmodium* parasite from the patient's bloodstream. Those without symptoms may be treated for infection to reduce the risk of disease transmission in the surrounding population. Artemisinin-based combination therapy (ACT) is recommended by the WHO to treat uncomplicated malaria. Artemisinin is derived from the plant *Artemisia annua*, better known as sweet wormwood. It is known for its ability to rapidly reduce the concentration of *Plasmodium* parasites in the bloodstream. ACT is artemisinin combined with a partner drug. The role of artemisinin is to reduce the number of parasites within the first 3 days of infection, while the partner drugs eliminate the rest. Expanding access to ACT treatment worldwide has helped reduce the impact of malaria, but the disease is becoming increasingly resistant to the effects of ACT. In places where malaria is resistant to ACT, treatment must contain an effective partner drug. The WHO has warned that no alternatives to artemisinin are likely to become available for several years.

#### **Descriptions of Specific Antimalarial Drugs**

**QUININE,** first isolated from cinchona bark in 1820, remains a fundamental tool for treating malaria, especially severe disease. Quinine acts rapidly, targeting the bloodborne asexual stages of all malaria species. It is available in oral and injectable preparations and can be used in infants and pregnant women. Side effects—nausea, mood change, blurred vision, and ringing in the ears—are common but typically resolve after treatment ends.

Since P. falciparum parasites from most areas of the world respond well to quinine, short courses of the drug are often sufficient when paired with a second drug. In Southeast Asia, however, full course quinine treatment is necessary, usually given in combination with a second drug such as tetracycline.

**CHLOROQUINE** is a 4-aminoquinoline derivative of quinine first synthesized in 1934. It is safe in infants and pregnant women, and was the historical drug of choice for treatment of nonsevere or uncomplicated malaria and to prevent malaria in travelers. Chloroquine acts primarily against bloodborne asexual stages, although it also works against the bloodstream stage infective to mosquitoes. Because of widespread resistance to this drug, its usefulness is increasingly limited. Side effects are uncommon and generally mild.

**AMODIAQUINE**, which is closely related to chloroquine, fell out of favor because it caused adverse effects on bone marrow and liver when used for prophylaxis. Amodiaquine is currently being reevaluated as a co-formulation partner with artesunate. Concerns over toxicity remain.

**ANTIFOL COMBINATION DRUGS** include various combinations of dihydrofolate reductase inhibitors (proguanil, chlorproguanil, pyrimethamine, and trimethoprim) and sulfa drugs (dapsone, sulfalene, sulfamethoxazole, sulfadoxine, and others). The partner drugs in antifol combinations have similar mechanisms of action; consequently, they do not protect each other from resistance to the same degree as unrelated drugs. Current combinations include sulfadoxine-pyrimethamine (SP; Fansidar), sulfalene/pyrimethamine (Metakelfin), and sulfamethoxazole/trimethoprim (cotrimoxazole). Proguanil has also been used in combination with chloroquine for prophylaxis in areas of moderate chloroquine resistance, although it confers only minimal added benefit, especially with prolonged exposure (Steffen *et al*., 1993). When used for prophylaxis, Fansidar can produce severe allergic reactions: in American travelers, Fansidar was linked to severe skin reactions (1 per 5,000 to 8,000 users) and mortality (1 per 11,000 to 25,000 users) (Miller *et al*., 1986). These adverse outcomes are not as frequent when a single dose of Fansidar is used for treatment. Concerns about sulfa drug use during pregnancy are outweighed by the known risks to mother and fetus of untreated malaria.

The latest antifol combination is chlorproguanil and dapsone, also known as Lapdap. This particular combination is inherently more effective than Fansidar (even in areas where resistance is present) and has a far shorter elimination time, which may decrease the likelihood of resistance (Watkins *et al*., 1997; Mutabingwa *et al*., 2001). On the other hand, its shorter half-life requires that Lapdap be given over 3 days rather than as SP’s one single dose.

**TETRACYCLINE** and derivatives such as doxycycline may be paired with other drugs for treatment or used as single agents for prophylaxis. In areas where quinine efficacy is diminished, tetracyclines are often added to quinine to improve cure rates. Tetracyclines are also used with shortened courses of quinine to decrease quinine-associated side effects. Tetracyclines are contraindicated in pregnant or breastfeeding women, or in children under age 8. Common side effects include nausea, vomiting, diarrhea, secondary yeast infections, and photosensitivity.

**PRIMAQUINE,** an 8-aminoquinoline, acts against malaria parasites in the liver, thereby reducing the likelihood of P. vivax or P. ovale relapse. Primaquine is also reasonably efficacious (74% efficacy against P. falciparum; 90 percent efficacy against P. vivax) when used for prophylaxis (Baird *et al*., 1995). Although it also has activity against blood-stage asexual parasites, drug concentrations that kill fully mature blood parasites are toxic. Primaquine is also a potent gametocidal drug, i.e., it kills the sexual stage of the malaria parasite infective to mosquitoes.

Primaquine can produce severe and potentially fatal hemolytic anemia in people with glucose-6-phosphate dehydrogenase (G6PD) enzyme deficiencies. The most severe Mediterranean B variant and related Asian variants of G6PD deficiency occur at high rates among several groups and regions: Kurdish Jews (62 percent), Saudia Arabia (13 percent), Burma (20 percent), and southern China (6 percent) and have now spread through migration and intermarriage. Primaquine should not be used in pregnancy.

**TAFENOQUINE,** a synthetic analog of primaquine, is currently being tested. It is highly effective against both liver and blood stages of malaria. Because of its long half-life (14 days versus 6 hours for primaquine), tafenoquine may prove to be a valuable chemoprophylactic drug (Lell *et al*., 2000). As with primaquine, tafenoquine can produce acute hemolytic anemia in patients with G6PD deficiency.

**MEFLOQUINE** is a quinoline-methanol derivative of quinine that can be used for treatment or prevention in most areas with multidrug resistant malaria. Resistance to mefloquine occurs frequently in parts of Southeast Asia, however, and sporadic resistance has been reported in areas of Africa and South America. Mefloquine causes a relatively high incidence of neuropsychiatric side effects when used at treatment doses and to a lesser degree when used for prophylaxis. In one large study in Asia, mefloquine was associated with stillbirth when given in pregnancy (Nosten *et al*., 1999).

**HALOFANTRINE** is a phenanthrene-methanol compound with activity against the bloodborne stages of the malaria parasite. It is especially useful in areas where multidrug-resistant falciparum malaria is present. Cardiac conduction abnormalities (specifically, prolongation of the PR and QT intervals on a standard electrocardiogram) are halofantrine’s major drawback (Nosten *et al*., 1993). Taking halofantrine immediately following mefloquine or quinine therapy also increases the risk of cardiac complications. Halofantrine and mefloquine may exhibit clinical cross-resistance (Wongsrichanalai *et al*., 1992; ter Kuile *et al*., 1993).

**CLINDAMYCIN** is an antibiotic with weak antimalarial activity. It should only be used in combination with a fast-acting schizonticide, such as quinine, especially when treating patients with little or no immunity to malaria (Pukrittayakamee *et al*., 2000b; Parola *et al*., 2001). Clindamycin is considered safe for use in pregnant women and very young children (Pukrittayakamee *et al*., 2000a).

**ARTEMISININ COMPOUNDS** include the compounds artesunate, artemether, arteether, and dihydroartemisinin derived from the sesquiterpene lactone principle (artemisinin) of the plant Artemisia annua. In severe malaria, artemisinin compounds produce faster parasite clearance and resolution of fever than quinine. Artemisinins also reverse coma more quickly than quinine (Taylor *et al*., 1993; Salako *et al*., 1994). However, used alone for periods under 5 to 7 days, recrudescence rates are high. For nonsevere malaria, artemisinins are most successful when used in combination with a second drug (Nosten *et al*., 1994). The best documented combination is mefloquine plus 3 days of artesunate.

The safety of artemisinins in early pregnancy is of particular concern since the drugs have produced fetal resorption in experimental animals. Despite reassuring clinical data on over 600 carefully followed pregnancies treated with artemisinins in the second and third trimesters of pregnancy, there are unresolved concerns about their effects in early human gestation.

A fixed-dose preparation of lumefantrine and artemether is commercially sold under the trade name of Coartem or Riamet. Lumefantrine (previously known as benflumetol) is an aryl-amino alcohol antimalarial compound. Although chemically related, lumefantrine does not appear to have the same cardiac effects as halofantrine (van Vugt *et al*., 1999).

Coartem is marketed in two packages: a six-dose (24-tablet) package intended for nonimmune patients and a four-dose (16-tablet) package for use by semi-immune patients. Until studies show conclusive efficacy of the four-dose regimen in semi-immune populations, all patients should receive the six-dose regimen (van Vugt *et al*., 2000). Until further safety data become available, lumefantrine is not recommended for treatment of pregnant women.

**ATOVAQUONE PLUS PROGUANIL (MALARONE)** is a fixed-dose combination containing 250 mg of atovaquone (a hydroxynaphthoquinone) and 100 mg of proguanil in a single adult-sized pill taken daily for prophylaxis. An adult treatment course of Malarone is 1,000 mg of atovaquone and 400 mg of proguanil daily for 3 days. Malarone has also been combined with artesunate for treatment of uncomplicated multidrug-resistant falciparum malaria (van Vugt *et al*., 2002). Malarone is also thought to be effective against bloodborne forms of P. vivax (Looareesuwan *et al*., 1996a).

**PYRONARIDINE** has been used in China for over 20 years. While it was reportedly 100 percent effective in a single trial in Cameroon, the drug was only 63 to 88 percent effective in Thailand (Ringwald *et al*., 1996; Looareesuwan *et al*., 1996b). Further testing is required before pyronaridine can be recommended for widespread use.

**PIPERAQUINE** is an orally active bisquinoline discovered in the early 1960s and developed for clinical use in China in 1973. In vitro testing in several laboratories has shown that piperaquine approximates chloroquine’s effects against sensitive parasites and is significantly more effective than chloroquine in treating resistant P. falciparum. In China, Vietnam and Cambodia, piperaquine is now available as a fixed combination with dihydroartemisinin (it has also been combined with trimethoprim and primaquine). Prospective clinical trial data are pending.

**2.1.9 Prevention**

There are several ways to keep malaria at bay.

There is no available vaccine for malaria at the moment.

* It is essential to seek medical attention for suspected symptoms of malaria as early as possible.
* Ensure there will be access to insect repellants, insecticides, pre-treated bed nets, and appropriate clothing and also be aware of the symptoms of malaria.

 **2.2** **Oxidative stress**

Oxidative stress, termed as an imbalance between production and elimination of reactive oxygen species (ROS) leading to plural oxidative modifications of basic and regulatory processes, can be caused in different ways. Increased steady-state ROS levels can be promoted by drug metabolism, overexpression of ROS-producing enzymes, or ionizing radiation, as well as due to deficiency of antioxidant enzymes. It is also a “state in which oxidation exceeds the antioxidant systems in the body secondary to a loss of the balance between them” (Wao *et al*.,2012).

Malaria infection induces the generation of hydroxyl radicals (OH) in the liver, which most probably is the main reason for the induction of oxidative stress. It was observed that erythrocytes infected with *P. falciparum* produced OH radicals and H2O2 about twice as much compared to normal erythrocytes. Higher level of this free radicals can lead to oxidative stress (Sandro *et al.,* 2012).

**2.2.1 Exogenous Source of Oxidants**

**1. Cigarette Smoke**: Cigarette smoke contains many oxidants and free radicals and organic compounds, such as superoxide and nitric oxide.( Church *et al*., 1985) In addition, inhalation of cigarette smoke into the lung also activates some endogenous mechanisms, such asaccumulation of neutrophils and macrophages, which further increase the oxidant injury.

**2. Ozone Exposure**  : This can cause lipid peroxidation and induce influx of neutrophils into the airway epithelium. Short-term exposure to ozone also causes the release of inflammatory mediators, such as MPO, eosinophil cationic proteins and also lactate dehydrogenase and albumin ( Hiltermann *et al*., 1999) Even in healthy subjects, ozone exposure causes a reduction in pulmonary functions. (Nightingale *et al*., 1999, Cho *et al*., 2005) have shown that particulate matter (mixture of solid particles and liquid droplets suspended in the air) catalyzes the reduction of oxygen.

 **3. Hyperoxia**: This is refers to conditions of higher oxygen levels than normal partial pressure of oxygen in the lungs or other body tissues. It leads to greater production of reactive oxygen and nitrogen species (Comhair *et al.,* 2000).

**4. Ionizing Radiation :** In the presence of O2, converts hydroxyl radical, superoxide, and organic radicals to hydrogen peroxide and organic hydroperoxides. These hydroperoxide species react with redox active metal ions, such as iron (Fe) and copper (Cu), via Fenton reactions and thus induce oxidative stress (Biaglow *et al*.,1992; Chiu *et al.,*1993) showed that fibroblasts that were exposed to alpha particles had significant increases in intracellular O2 and H2O2 production via plasma membrane-bound NADPH oxidase (Narayanan *et al*., 1997). Signal transduction molecules, such as extracellular signal-regulated kinase 1 and 2 (ERK1/2), c-Jun N-terminal kinase (JNK), and p38, and transcription factors, such as activator protein-1 (AP-1), nuclear factor-kB (NF-kB), and p53, are activated, which result in the expression of radiation response–related genes.(Tuttle *et al.,*1992; Wei *et al*., 2000) Ultraviolet A (UVA) photons trigger oxidative reactions by excitation of endogenous photosensitizers, such as porphyrins, NADPH oxidase, and riboflavins. 8-Oxo-7,8- dihydroguanine (8-oxoGua) is the main UVA-mediated DNA oxidation product formed by the oxidation of OH radical, 1-electron oxidants, and singlet oxygen that mainly reacts with guanine.(Cadet *et al*.,2003) The formation of guanine radical cation in isolated DNA has been shown to efficiently occur through the direct effect of ionizing radiation (Yokoya *et al*., 2002; Janssen *et al.,*1993). After exposure to ionizing radiation, intracellular level of ROS generated by metal-catalyzed reactions can modify DNA bases. Three base substitutions, G / C, G / T, and C / T, can occur as a result of oxidative damage by metal ions, such as Fe2+, Cu2+, and Ni2+. (Reid *et al*., 1994) showed that G / C was predominantly produced by Fe2+ while C / T substitution was by Cu2+ and Ni2+.

#### **5. Heavy Metal Ions:**  Heavy metal ions, such as iron, copper, cadmium, mercury, nickel, lead, and arsenic, can induce generation of reactive radicals and cause cellular damage via depletion of enzyme activities through lipid peroxidation and reaction with nuclear proteins and DNA (Stohs et al., 1995).

**2.2.2 Consequences of Oxidative stress**

 Oxidative stress does not only cause hazardous events such as lipid peroxidation and oxidative DNA damage, but also physiologic adaptation phenomena and regulation of intracellular signal transduction. The consequence of oxidative stress once it is high, it can cause damage to the brain, metabolic disorders or inherited disease affecting electron transport chain. From a clinical standpoint, if biomarkers that reflect the extent of oxidative stress were available, such markers would be useful for physicians to gain an insight into the pathological features of various diseases and assess the efficacy of drugs.

####  **2.2.3. Oxidative Stress and the Membrane of Infected-Erythrocyte**

During the development of the blood *esquizogeny*, *P. falciparum* trophozoites increase the viscosity of red blood cells by causing changes in the parasitized cell surface permitting its adhesion to the endothelial wall of capillaries, which seems to be a defense mechanism of the parasite, preventing the passage of parasitized red blood cells through the spleen and their consequent destruction (Luse *et al*., 1971). However, the increased viscosity of the cells appears to be primarily responsible for the blocking of blood vessels, especially of kidney capillaries, pulmonary capillaries and brain capillaries, and cerebral malaria is the most common reason for coma and death in infected children (Braga *et al*., 2005; Phiri *et al*., 2009).

Among the changes that take place on the surface of red blood cells is the phenomenon of lipid peroxidation. In this sense, the parasitized erythrocytes are known to contain large amounts of monohydroxy derivatives of polyenoic fatty acids (OH-PUFA) in their lipids, suggesting the occurrence of lipid peroxidation due to the release of heme iron from non-enzymatic breakdown (Schwarzer *et al*., 2003). One of the common OH-PUFA found and already described as toxic is the 12- and 15-hydroxy-arachidonic acid (HETE). It is known that the concentrations of OH-PUFA increase according to the evolutionary stage of the parasite. However, low concentrations of HETE were found after phagocytosis of parasitized RBCs, suggesting that other lipid peroxidation products also may play a key role in this process (Schwarzer *et al*., 2003).

Additionally, oxidative changes in *P. falciparum*-infected red blood cells seem to be associated with the accelerated aging of these cells and contribute to the development of anaemia presented by these subjects (Omodeo-sale *et al*., 2003). The development of anaemia can promote changes in the circulatory physiology, leading to the existence of moments of hypoxia alternating with the maintenance of tissue oxygenation at basal levels, favouring the participation of ischaemia and reperfusion syndrome (IRS) responsible for an additional production of free radicals (Halliwell *et al*., 2007).

Indeed, increased lipid peroxidation and oxidative stress reported in human malaria can affect the membrane of infected erythrocytes, also promoting the reduction of the deformity of these cells, which has been linked to increased mortality of adults and children with malaria. The deleterious consequences of increased cell rigidity include microcirculatory obstruction (exacerbating tissue hypoperfusion) and cell stiffness with subsequent removal by the spleen, which increases anemia (Becker *et al*., 2004).

**2.4 ANTIOXIDANTS**

Antioxidants are molecules that inhibit or quench free radical reactions and delay or inhibit cellular damage (Young *et al*., 2001). Though the antioxidant defenses are different from species to species, the presence of the antioxidant defense is universal. . Antioxidants exists both in enzymatic and non-enzymatic forms in the intracellular and extracellular environment. . Enzymatic antioxidants work by breaking down and removing free radicals. The antioxidant enzymes convert dangerous oxidative products to hydrogen peroxide (H2O2) and then to water, in a multi-step process in presence of cofactors such as copper, zinc, manganese, and iron. Non-enzymatic antioxidants work by interrupting free radical chain reactions (Young *et al*.,2001).

**2.4.1 Enzymatic Antioxidants:**  The major enzymatic antioxidants of the lungs are Superoxde Dismutase (SODs), Catalase, and Glutathione Peroxidase (GSH-Px). In addition to these major enzymes, other antioxidants, including heme oxygenase-1, and redox proteins, such as thioredoxins, peroxiredoxins , and glutaredoxins, have also been found to play crucial roles in the pulmonary antioxidant defenses. Since superoxide is the primary ROS produced from a variety of sources, its dismutation by SOD is of primary importance for each cell. All 3 forms of SOD, that is, CuZnSOD, Mn-SOD, and EC-SOD, are widely expressed in the human lung. Mn-SOD is localized in the mitochondria matrix. EC-SOD is primarily localized in the extracellular matrix, especially in areas containing high amounts of type I collagen fibers and around pulmonary and systemic vessels. It has also been detected in the bronchial epithelium, alveolar epithelium, and alveolar macrophages (Kinnula *et al*., 2003; Kinnula ,2005). Overall, CuZnSOD and Mn-SOD are generally thought to act as bulk scavengers of superoxide radicals. The relatively high EC-SOD level in the lung with its specific binding to the extracellular matrix components may represent a fundamental component of lung matrix protection. H2O2 that is produced by the action of SODs or the action of oxidases, such as xanthine oxidase, is reduced to water by catalase and the GSH-Px. Catalase exists as a tetramer composed of 4 identical monomers, each of which contains a heme group at the active site. Degradation of H2O2 is accomplished via the conversion between 2 conformations of catalase-ferricatalase (iron coordinated to water) and compound I (iron complexed with an oxygen atom). Catalase also binds reduced Nicotinamide adenine dinucleotide phosphate (NADPH) as a reducing equivalent to prevent oxidative inactivation of the enzyme (formation of compound II) by H2O2 as it is reduced to water (Kirkman *et al*., 1999). Enzymes in the redox cycle responsible for the reduction of H2O2 and lipid hydroperoxides (generated as a result of membrane lipid peroxidation) include the GSH-Pxs (Flohe 1988). The GSH-Pxs are a family of tetrameric enzymes that contain the unique amino acid selenocysteine within the active sites and use low-molecular-weight thiols, such as glutathione (GSH), to reduce H2O2 and lipid peroxides to their corresponding alcohols. Four GSHPxs have been described, encoded by different genes: GSHPx-1 (cellular GSH-Px) is ubiquitous and reduces H2O2 and fatty acid peroxides, but not esterified peroxyl lipids (Arthur 2000). Esterified lipids are reduced by membrane-bound GSH-Px-4 (phospholipid hydro peroxide GSH-Px), which can use several different low-molecular-weight thiols as reducing equivalents. GSH-Px-2 (gastrointestinal GSH-Px) is localized in gastrointestinal epithelial cells where it serves to reduce dietary peroxides (Chu *et al*., 1993). GSH-Px-3 (extracellular GSH-Px) is the only member of the GSH-Px family that resides in the extracellular compartment and is believed to be one of the most important extracellular antioxidant enzyme in mammals. Of these, extracellular GSH-Px is most widely investigated in the human lung (Comhair *et al*., 2001). In addition, disposal of H2O2 is closely associated with several thiol-containing enzymes, namely, TRXs (TRX1 and TRX2), thioredoxin reductases (TRRs), PRXs (which are thioredoxin peroxidases), and glutaredoxins. (Gromer *et al*., 2004) Two TRXs and TRRs have been characterized in human cells, existing in both cytosol and mitochondria. In the lung, TRX and TRR are expressed in bronchial and alveolar epithelium and macrophages. Six different PRXs have been found in human cells, differing in their ultrastructural compartmentalization. Experimental studies have revealed the importance of PRX VI in the protection of alveolar epithelium. Human lung expresses all PRXs in bronchial epithelium, alveolar epithelium, and macrophages (Kinnula *et al*., 2002). PRX V has recently been found to function as a peroxynitrite reductase (Dubuisson *et al*., 2004). Which means that it may function as a potential protective compound in the development of ROS-mediated lung injury (Holmgren, 2000). Common to these antioxidants is the requirement of NADPH as a reducing equivalent. NADPH maintains catalase in the active form and is used as a cofactor by TRX and GSH reductase , which converts GSSG to GSH, a co-substrate for the GSH-Pxs. Intracellular NADPH, in turn, is generated by the reduction of NADP+ by glucose-6-phosphate dehydrogenase, the first and rate-limiting enzyme of the pentose phosphate pathway, during the conversion of glucose6-phosphate to 6-phosphogluconolactone. By generating NADPH, glucose-6-phosphate dehydrogenase is a critical determinant of cytosolic GSH buffering capacity (GSH/ GSSG) and, therefore, can be considered an essential, regulatory antioxidant enzyme (Dickinson *et al*., 2002; Sies *et al*., 1999). Glutathione S-transferases (GSTs), another antioxidant enzyme family, inactivate secondary metabolites, such as unsaturated aldehydes, epoxides, and hydroperoxides. Three major families of GSTs have been described: cytosolic GST, mitochondrial GST, (Ladner *et al*., 2004; Robinson *et al*., 2004) and membrane-associated microsomal GST that has a role in eicosanoid and GSH metabolism (Jakobsson *et al*., 1999).

**2.4.2 Non-enzymatic Antioxidants:** This include low-molecular-weight compounds, such as vitamins (vitamins C and E), β-carotene, uric acid, and GSH, a tripeptide (L-γ-glutamyl-L-cysteinyl-Lglycine) that comprise a thiol (sulfhydryl) group. Vitamin C (Ascorbic Acid) Water-soluble vitamin C (ascorbic acid) provides intracellular and extracellular aqueous-phase antioxidant capacity primarily by scavenging oxygen free radicals. It converts vitamin E free radicals back to vitamin E. Its plasma levels have been shown to decrease with age. (Bunker., 1992, Mezzetti *et al*., 1996) Vitamin E (α-Tocopherol) Lipid-soluble vitamin E is concentrated in the hydrophobic interior site of cell membrane and is the principal defense against oxidant-induced membrane injury. Vitamin E donates electron to peroxyl radical, which is produced during lipid peroxidation. a-Tocopherol is the most active form of vitamin E and the major membrane-bound antioxidant in cell. Vitamin E triggers apoptosis of cancer cells and inhibits free radical formations (White *et al*., 1997). Glutathione GSH is highly abundant in all cell compartments and is the major soluble antioxidant. GSH/GSSG ratio is a major determinant of oxidative stress. GSH shows its antioxidant effects in several ways (Masella *et al*., 2005). It detoxifies hydrogen peroxide and lipid peroxides via action of GSH-Px. GSH donates its electron to H2O2 to reduce it into H2O and O2. GSSG is again reduced into GSH by GSH reductase that uses NADPH as the electron donor. GSH-Pxs are also important for the protection of cell membrane from lipid peroxidation. Reduced glutathione donates protons to membrane lipids and protects them from oxidant attacks (Curello *et al*., 1985). GSH is a cofactor for several detoxifying enzymes, such as GSH-Px and transferase. It has a role in converting vitamin C and E back to their active forms. GSH protects cells against apoptosis by interacting with proapoptotic and antiapoptotic signaling pathways (Masella *et al*., 2005). It also regulates and activates several transcription factors, such as AP-1, NF-kB, and Sp-1. Carotenoids (b-Carotene) Carotenoids are pigments found in plants. Primarily, b-carotene has been found to react with peroxyl (ROO), hydroxyl (OH), and superoxide radicals (El-Agamey *et al*., 2004). Carotenoids show their antioxidant effects in low oxygen partial pressure but may have pro-oxidant effects at higher oxygen concentrations. (Rice-Evans *et al*., 1997) Both carotenoids and retinoic acids (RAs) are capable of regulating transcription factors (Niles, 2004). β-Carotene inhibits the oxidant-induced NF-kB activation and interleukin (IL)-6 and tumor necrosis factor-a production. Carotenoids also affect apoptosis of cells. Antiproliferative effects of RA have been shown in several studies. This effect of RA is mediated mainly by retinoic acid receptors and vary among cell types. In mammary carcinoma cells, retinoic acid receptor was shown to trigger growth inhibition by inducing cell cycle arrest, apoptosis, or both (Donato *et al*., 2005; Niizuma *et al*., 2006).

**2.4 Glucose 6 phosphate dehydrogenase**

Glucose – 6 – phosphate dehydrogenase (G6PD) is a highly conserved housekeeping enzymes and rate – limiting enzyme of the pentose phosphate pathway in all cells (Cappellini and Fiorelli, 2008). The pentose phosphate pathway (PPP) convert glucose to ribose -5-phosphate, a precursor to RNA, DNA, ATP, CoA, NAD, and FAD. During this reaction, NADPH is produced and protects red blood cells from oxidative damage. In mammalian cells G6PD provides reductive potential in the form of NADPH (Chanmugam and Frumin, 1964). G6PD is a ubiquitous enzyme that must be quite ancient in evolution because it has been found in all organisms, from prokaryotes to yeast, protozoa, plants and animals (Donde *et al*., 1985, Mehta *et al*., 2000).

G6PD deficiency result from mutation in the G6PD gene and is well- known common causes of hemolytic anemia in human (Halliwell and Gutteridge, 1989). Most cells have a back-up system of other metabolic pathways that can generate the intracellular NADPH necessary, but red blood cells do not have other NADPH producers. Therefore, G6PD deficiency becomes especially lethal in red blood cells, where any oxidative stress will result in hemolytic anemia.G6PD deficiency was first identified in American blacks in the course of studies of sensitivity to the hemolytic effect of worldwide (Beutler, 1994). Clinically, this deficiency affects as many as 400 million individuals infection-mediated hemolytic crisis, fauvism and less commonly to chronic nonspherocytic hemolytic anemia (Moussa, 2008).

**2.4.1 Function of Glucose-6- Phosphate Dehydrogenase**

G6PD catalyzes the first step in the pentose phosphate pathway, converts Glucose-6-phosphate into 6-phosphogluconolactone and during this conversion, the important reductant metabolite named NADPH is provided. The pathway is the only source for producing of NADPH in red blood cells (Halliwell and Gutteridge, 1999), because they lack mithochondria, nucleus and ribosomes and other pathways that produce NADPH (Bell., 2004). NADPH is necessary for generating of GSH fromits oxidized form, GSSG, and subsequent maintenance of intracellular GSH pools. GSH maintains normal structure, elasticity and integrity of red blood cells, and sustain hemoglobin in ferrous state that is essential for carrying oxygen (Bell, 2004). G6PD and NADPH are key factors for protection of red blood cells from oxidative damage and peroxides (Halliwell and Gutteridge., 1999). Peroxides are usually removed from red blood cells by glutathione peroxidase that uses reduced glutathione. Reduced glutathione reacts with harmful peroxides and neutralize them. In this reaction reduced glutathione is oxidized and glutathione reductase regenerates reduced glutathione by using of NADPH. NADPH is oxidized and G6PD is required for producing reduced NADPH again (Valko *et al*., 2006). Catalase is another anti-oxidant enzyme that is abundant in red blood cells and helps to the removal of peroxides from red blood cells through activation by NADPH (Halliwell and Gutteridge., 1999). Unless peroxides are neutralized, they will cause oxidative injuries. Hemoglobin and red blood cell membrane molecules that contain SH groups are destroyed (Valko *et al*., 2006). Hemoglobin is denatured irreversibly, precipitates and forms Heinz bodies. Heinz bodies destroy membranes of red blood cells then leads to haemolysis and acute anemia. Regarding to the roles of NADPH in redox state of cells, G6PD is the principal enzyme in chain reaction that is necessary for protecting all cells especially red blood cells against oxidant agents. Then G6PD-deficient cells especially red blood cells are susceptible to damage by reactive oxygen species and oxidative stress (Halliwell and Gutteridge., 1999).

**2.4.2 Glucose 6 phosphate dehydrogenase deficiency**

 G6PD deficiency is an inherited disorder caused by a defect or deficiency in the production of that enzyme. Consequently, G6PD deficiency may cause the destruction of RBCs leading to a condition known as haemolytic anemia in which the body cannot compensate for the destroyed cells. In this condition, the patient may show jaundice (pallor, yellowing of the skin, and discoloration of the eyes), dark urine, fatigue, shortness of breath, and a rapid heart rate. Many patients, however, remain without symptoms. Sometimes severe hemolytic crisis in individuals with G6PD deficiency require immediate medical interventions (Gomez-Gallego, 2000).

G6PD deficiency is the most common human blood enzyme deficiency affecting about 400 million people worldwide. The highest prevalence rates occur in areas where malaria is common, such as: tropical Africa (20% of the population are affected), the Mediterranean (4-30% are affected), tropical and subtropical Asia, and Papua New Guinea. However, the severity of the disease varies among populations with the milder form being common in Africans, while the most severe form is found in Mediterraneans and South East Asians (Nkhoma *et al*., 2009).

**2.5 Malaria and G6PD deficiency**

The geographical distribution of malaria is similar to the world distribution of deficient G6PD variants. It is postulated that the high frequency of G6PD deficiency has arisen because G6PD deficient variants confer some resistance against severe malaria caused by *Plasmodium* *falciparum* (Siems *et al., 2004*; Asami *et al*., 1997; Andreadis *et al*., 2003). The exact mechanism of this protection is still unknown. Red blood cells are the host cells for *Plasmodium falciparum*. *Plasmodium* parasites oxidize NADPH and diminish the level of reduced glutathione (GSH) in red blood cells. This effect in G6PD deficiency is more severe, leading to oxidative-induced damage to the RBC (Siems *et al* 1995). Also *Plasmodium* parasites break down haemoglobin, and release toxic substances like Fe, which is a source of oxidative stress and it will cause haemolysis (Comhair *et al.,* 2005).

**CHAPTER THREE**

**3.0 Materials and Methods**

**3.1 Reagent and apparatus/equipment for laboratory analysis**

**3.1.1 Consumables**

* micropipette tips (50µl, 200µl and 1000µl)
* Cotton wool
* Hand gloves
* Test strips for glucometer
* Methylated spirit
* Eppendoff tubes (1.5ml)
* Non EDTA tubes
* EDTA tubes

**3.1.2** **Reagents**

* 0.25M Hydrochloric acid
* 0.18M Sodium nitrate solution
* 15% Trichloroacetic acid
* 0.28M Glucose solution
* 0.01M Phosphate buffer pH7.0
* 0.37% Thiobarbituric acid
* 1M Phosphate buffer pH7.0
* 0.2M Hydrogen peroxide
* 5% Potassium dichromate (VI) (K2Cr2O7) in acetic acid
* 0.0004M Methylene blue
* 0.6M Potassium ferricyanide
* 0.4M Sodium cyanide

**3.1.3 Equipments**

* Centrifuge (Model 800D, England)
* Water Bath (Model DK England)
* Electrical Weighing Balance (Model no:Yp.502N)
* Spectrophotometer (spectrum lab 23A,England)
* Adjusted Micropipette (Perfect,U.S.A)
* Refrigerator (Kelvinator,Germany)
* Beakers
* Reagent bottles
* Stirring rods
* Pipette pumps
* Spatula
* Volumetric Flask
* Conical flasks

**3.2 Study Population:**

* **Study duration:** This one month study was carried out between May to early June 2018 at Enugu University of Science and Technology Teaching Hospital, Parklane, Enugu.
* **Ethical consideration:** Ethical approval was obtained from the institutional review board of ESUT teaching hospital Parklane gave the approval number: ESUT P/C-MAC/RA/034/Vol.11/35 and written informed consent was obtained from willing participants.
* **Data collection:** After written informed consent was obtained from subjects, a questionnaire was used to provide basic information of patients including their names, age, sex, familiar history of diabetes, ethnic group, state and country of origin.
* **Measurement of blood pressure:** The patients systolic and diasystolic blood pressure were measured using an automatic sphygmomanometer and their height as well as their weight was also measured.
* **Blood collection:** Five milliliters of blood samples were drawn by venipuncture technique from patients into EDTA tubes and were properly swirled to avoid clotting. Also, a volume of 5ml of blood sample was used for the determination of malaria, protein oxidation, lipid oxidation and G6PD deficiency. This sample was collected ones an interaction is reached with the patient through the filling of the consent form. Collection of samples and data lasted for 20 minutes.

**3.3 PROCEDURES**

**3.3.1 Determination of Malaria Parasite**

1. **Microscopy:** This method involves thick and thin blood smear study regarded as the gold standard method for malaria diagnosis. The procedure used for this test includes the collection of the peripheral blood, staining of smear with Giemsa stain for about 60 seconds and examination of the red blood cells for malaria parasites under the microscope
2. **Procedure for Giemsa Method**

1. Estimate the amount of 10% Giemsa working solution required for the number of slides to be stained. Each slide requires approximately 3 mL of stain to cover it. Prepare the stain immediately before use according to MM-SOP-04: Preparation of Giemsa working solution.

 2. To fix the thin film, preferably use a Pasteur pipette or dip the thin film for 2 s into a small container or beaker containing methanol. Avoid contact between the thick film and methanol, as methanol and its vapours will quickly fix the thick film and interfere with haemolysis of the thick film.

 3. Place the slides on a tray or drying rack. Allow the methanol-fixed thin smear to dry completely in air (approximately 2 min) by placing the slides on a flat surface. Never let the slide dry in a vertical position with the thin film down, as this may lead to fixing of the thick film by methanol vapour.

 4. Place slides for staining blood films face down if using a curved staining tray or facing up if using a staining rack.

 5. Pour the stain gently between the slide and the staining tray if staining face down, until each slide is covered with stain, or gently pour the stain onto the top of slides lying face upwards on a staining rack. 6. Set the timer to 8–10 min (the exposure time should be determined previously by testing the batch of stock staining solution used), and allow the blood films to stain. Experience with the stain you are using will help indicate the time required for good staining. See MMSOP 3c: Quality control of Giemsa stock solution and buffered water.

 7. At the end of the staining time, remove each slide individually. Gently flush the stain from the slide by adding drops of buffered water until all the stain has been washed away. Do not pour the stain directly off the slides, as the metallic green surface scum will stick to the film, spoiling it for microscopy.

 8. When the stain has been washed away, place the slide in the drying rack film side downwards, or in a vertical position with the thick film down to drain and dry. Ensure that thick films are not scraped against the edge of the rack.

 9. Discard the remaining 10% Giemsa solution.

**PRINCIPLE/ PROCEDURE OF RAPID DIAGNOSED TEST (RDT)**

 **PRINCIPLE**

RDTs for the detection of malaria antigens are based on the immunochromatographic test principle. These RDTs capture parasite antigen from peripheral blood using monoclonal antibodies prepared against a malaria antigen target and conjugated to gold particles in a mobile phase.  Test area contains immobilized monoclonal antibody, which captures the Ag-Ab complex giving a visible line.

If malaria antigen is present in the blood sample, Ag-Ab complex will be formed as it combines with the labeled pan-specific antibody present in the mobile phase. This Ag-Ab complex will migrate along the test strips, which will be captured by the specific antibodies present in the immobile phase *(here T1 contains monoclonal antibodies specific to P.falciparum i.e. HRP2 antigen and T2 contains plasmodium pan specific antibody i.e. pLDH)*thus producing a visible colored line.  Control line contains goat anti-mouse antibody and ensures that system is controlled for migration.

Malaria antigens currently targeted by RDT are HRP-2, pLDH, and Plasmodium aldolase.

**HRP-2** : HRP-2 is a water-soluble protein produced by asexual stages and young gametocytes of *Plasmodium falciparum*. It is expressed on the RBC membrane surface, and because of its abundance in *P.falciparum*, it is the first antigen to be used to develop an RDT for its detection.

**PROCEDURE**

1. Check the expiry date on the test packet.
2. Put on the gloves. Use new gloves for each patient.
3. Open the test kit packet and remove, test pad, capillary tube and Desiccant sachet.
4. Write the patient’s name on the test.
5. Open the alcohol swab. Grasp the 4th finger on the patient’s left hand. Clean the finger with the alcohol swab. Allow the finger to dry before pricking.
6. Open the lancet. Prick patient’s finger to get a drop of blood. Do not allow the tip of the lancet to touch anything before pricking the patient’s finger.
7. Discard the lancet in the Sharps Box immediately after pricking finger. Do not set the lancet down before discarding it.****
8. Use the capillary tube to collect the drop of blood.
9. Use the capillary tube to put the drop of blood into the square hole marked “A”.
10. Discard the capillary tube in the Sharps Box.
11. Add buffer into the round hole marked “B”.
12. **Wait for 15 minutes after adding buffer.**
13. Read the results (see below)
14. Dispose of the gloves, alcohol swab, desiccant sachet and packaging in a non-sharps waster container.
15. Record the test results in your register. Dispose of cassette in non-sharps waste container.

**ANAEMIA**

Anaemia is describes the condition in which the number of red blood cells in the blood is low

 **Common causes of anemia include the following:**

* **Anaemia from active bleeding:** Loss of blood through heavy menstrual bleeding or [wounds](https://www.emedicinehealth.com/wound_care/article_em.htm) can cause anaemia. [Gastrointestinal](https://www.emedicinehealth.com/tummy_trouble_quiz_iq/quiz.htm) ulcers or cancers such as [cancer of the colon](https://www.emedicinehealth.com/colon_cancer/article_em.htm) may slowly ooze blood and can also cause anaemia.
* **Iron deficiency anaemia:** The bone marrow needs iron to make red blood cells. Iron (Fe) plays an important role in the proper structure of the haemoglobin molecule. If iron intake is limited or inadequate due to poor dietary intake, anaemia may occur as a result. This is called [iron deficiency anaemia](https://www.emedicinehealth.com/blood_and_bleeding_disorders_quiz_iq/quiz.htm). Iron deficiency anaemia can also occur when there are stomach ulcers or other sources of slow, chronic bleeding ([colon cancer](https://www.emedicinehealth.com/colon_cancer/article_em.htm), uterine [cancer](https://www.emedicinehealth.com/cancer_what_you_need_to_know/article_em.htm), intestinal polyps, [haemorrhoids](https://www.emedicinehealth.com/hemorrhoids/article_em.htm), etc). In these kinds of scenarios, because of ongoing, chronic slow blood loss, iron is also lost from the body (as a part of blood) at a higher rate than normal and can result in iron deficiency anaemia.
* **Anaemia of chronic disease:** Any long-term medical condition can lead to anaemia. The exact mechanism of this process in unknown, but any long-standing and ongoing medical condition such as a chronic infection or a [cancer](https://www.emedicinehealth.com/slideshow_pictures_cancer_101/article_em.htm) may cause this type of anaemia.
* **Anaemia related to kidney disease:** The kidneys release a hormone called the erythropoietin that helps the bone marrow make red blood cells. In people with chronic (long-standing) [kidney disease](https://www.emedicinehealth.com/kidney_disease_quiz_iq/quiz.htm) ([CKD](https://www.emedicinehealth.com/chronic_kidney_disease/article_em.htm) or [end stage renal disease](https://www.emedicinehealth.com/chronic_kidney_disease/article_em.htm) ([ESRD](https://www.emedicinehealth.com/kidney_disease_quiz_iq/quiz.htm)), the production of this hormone is diminished, and this, in turn, diminishes the production of red blood cells, causing anemia. This is called anemia related to or anaemia of chronic kidney disease.
* **Anaemia related to pregnancy:** Water weight and fluid gain during [pregnancy](https://www.emedicinehealth.com/pregnancy_week_by_week/article_em.htm) dilutes the blood, which may be reflected as anaemia since the relative concentration of red blood cells is lower.
* **Anaemia related to poor nutrition:** [Vitamins](https://www.emedicinehealth.com/vitamins_and_supplements_quiz_iq/quiz.htm) and minerals are required to make red blood cells. In addition to iron, [vitamin B12](https://www.emedicinehealth.com/drug-cyanocobalamin_injection/article_em.htm) and folate (or [folic acid](https://www.emedicinehealth.com/drug-folic_acid/article_em.htm)) are required for the proper production of haemoglobin (Hgb). Deficiency in any of these may cause anaemia because of inadequate production of red blood cells. Poor dietary intake is an important cause of low folate and low vitamin B12 levels. Strict vegetarians who do not take sufficient vitamins are at risk to develop vitamin B12 deficiency.
* **Pernicious anaemia:** There also may be a problem in the stomach or the [intestines](https://www.emedicinehealth.com/image-gallery/intestines_picture/images.htm) leading to poor absorption of vitamin B12. This may lead to anemia because of vitamin B12 deficiency known as [pernicious anaemia](https://www.emedicinehealth.com/image-gallery/pernicious_anemia_picture/images.htm).
* **Sickle cell anaemia:** In some individuals, the problem may be related to production of abnormal haemoglobin molecules. In this condition, the hemoglobin problem is qualitative, or functional. Abnormal haemoglobin molecules may cause problems in the integrity of the red blood cell structure and they may become crescent-shaped (sickle cells). There are different types of [sickle cell anaemia](https://www.emedicinehealth.com/sickle_cell_crisis/article_em.htm) with different severity levels. This is typically hereditary and is more common in those of African, Middle Eastern, and Mediterranean ancestry. People with sickle cell anaemia can be diagnosed as early as childhood depending on the severity and symptoms of their disease.
* **HAEMOGLOBIN:** Is a protein contained in red blood cells which carries oxygen.
* **Procedure for Haemoglobin and Haematocrite:**
* Insert the haemoglobin strip into the hemoglobinometer then, Take blood in the haemoglobin pipette up to 20-cubic-mm-mark and blow it into hemoglobinometer After 10 minutes ,then read the result.

|  |
| --- |
| **WHO's Hemoglobin thresholds used to define anemia**[[39]](https://en.wikipedia.org/wiki/Anemia#cite_note-39)**(1 g/dL = 0.6206 mmol/L)** |
| **Age or gender group** | **Hb threshold (g/dl)** | **Hb threshold (mmol/l)** |
| Children (0.5–5.0 yrs) | 11.0 | 6.8 |
| Children (5–12 yrs) | 11.5 | 7.1 |
| Teens (12–15 yrs) | 12.0 | 7.4 |
| Women, non-pregnant (>15yrs) | 12.0 | 7.4 |
| Women, pregnant | 11.0 | 6.8 |
| Men (>15yrs) | 13.0 | 8.1 |

ssSource:https:// en.m.wikipedia.org/wiki/anaemia.

**3.3.5 Determination of Glucose 6 Phosphate (G6PG) Status**

G6PG deficiency was assayed by the methaemoglobin reduction test of Brewer *et al.* (1960) with slight modifications. Blood sample (500 µl) collected in EDTA tubes and 50 µl of 0.28M of glucose was added to a set of three tubes labeled as Test, Deficient-Control and Normal-Control. Sodium nitrite (0.18 M, 50 µl) and methylene blue (0.4mM, 50 µl) solutions was transferred into the tubes labeled “Test”. A volume of sodium nitrite (50 µl) and normal saline (50 µl) solutions were transferred to the tubes labeled “Normal-Control”. All of the tubes were well mixed, corked with cotton wool and then incubated at 37oC for 3 hours. After incubation, 100 µl of the incubated mixture was transferred to new set of tubes labeled as before, the 5 ml of 0.02M (PH 6.6) phosphate buffer was added and colour visualized. A dark brown or grey is indicative of G6PD deficient while a red colour like the Normal-Control is considered as Non-deficient.

**3.3.3 Estimation of Lipid Peroxidation (Malondiadehyde)**

The method of Niehaus and Samuelsson (1968) was adopted for the determination of lipid peroxidation in blood with slight modifications. This involves a colorimetric assay for the quantification of thiobarbituric acid reactive substances. Hundred microlitre (100 µl) of whole blood (10 % w/v) was transferred to a 2ml mixtures (1:1:1 ratio) of thiobarbituric acid (0.37%), 15% trichloroacetic acid and 0.25 mol/l hydrogen chloride reagent. The resultant solution was incubated in a water bath at 100oC for 30 minutes after which it was cooled. A clear supernatant was obtained and the absorbance was read at 535 nm against the blank to quantify the amount of Malondialdehyde (MDA) formed. The MDA concentration will be determined using the formula:

C = A / E × L

Where, A is the sample absorbance; E is the extinction coefficient (1.56 × 105 M-1 cm-1); L is the light path length (1cm).

**3.3.4 Determination of Protein Oxidation**

The carbonyl content of protein was quantified according to the method of Mesquita *et al.* (2014). Blood sample (400 µl) was added to (400 µl) of 10 mM 2,4-Dinitrophenylhydrazine (DNPH) prepared in 0.5 M metaphosphoric acid (H3PO4) and was incubated at room temperature for 10 minutes. This was followed by the addition of 200 µl of 6 M Sodium Hydroxide, to the solution and incubated for 10 minutes at room temperature. The absorbance was taken at 450 nm against the blank where the sample solution was substituted by an equal volume of distilled water.

The concentration of carbonyl content was calculated using the equation:

C = A / E × L

Where, A is the sample absorbance; E is the extinction coefficient (22,000 M-1 cm-1); L is the light path length (1cm).

**3.3.8 Statistical analysis**

 Data was analyzed using statistical package for social sciences (SPSS) version 16. Genotype and allele frequencies were compared using X2 statistics or the fisher’s exact test. Continuous variables were compared using parametric tests independent sample t-test. Interaction between study parameters was done by multivariate analysis using 2 way ANOVA. A confidence interval of 95% was taken and a *p*-value less than 0.05 was considered to be statistically significant.

**CHAPTER FOUR**

**4.0 RESULTS**

**Table 1: Gender distribution of study population**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Characteristics(n = 101) |  | Malaria patients | Non-malariaPatients |  | *χ2* | *p-value* |
| Study participants | *Total* | 86 (85.1%) | 15 (14.9%) | 101 (100% |  |  |
| Sex | *Male* | 24 (23.8%) | 6 (5.9%) | 30 (29.7%) | 0.048 | 0.827 |
|  | *Female* | 62 (61.4%) | 9 (8.9%) | 71 (69.3%) |  |  |

**Table 2: Comparison between RDT and Microscopy techniques in diagnosing Malaria**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Study participants |  | Microscopy |  |  | *χ2* | *p-value* |
|  |  | **Positive** | **Negative** | Total |  |  |
|  | Positive | 9 (8.9%) | 0 (0.0%) | 9 (8.9%) |  |  |
| RDT  | Negative | 77 (76.2%) | 15 (14.9%) | 92 (91.1%) | *1.723* | 0.189 |
|  | Total | 86 (85.1%) | 15 (14.9%) | 101 (100.0%) |  |  |

**Table 3: Performance of RDT kits in diagnosing malaria using microscopy as standard**

|  |  |  |  |
| --- | --- | --- | --- |
|  |  | ELISA as Gold Standard |  |
|  | **Sensitivity (%)** | **Specificity (%)** | **PPV (%)** | **NPV (%)** | **Accuracy (%)** |
| RDT | 10.47 | 100 | 100 | 16.30 | 23.76 |

**Table 4: Baseline Characteristics of study participants**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Characteristics (n =101) | Malaria patients | Non-malariaPatients | Minimum | Maximum | *p-value* |
| Age (years) | 34.54±1.91 | 37.27±4.28 | 3 | 81 | 0.572 |
| Height (m) | 8.60±2.95 | 5.65±0.19 | 0.8 | 1.62 | 0.701 |
| Weight (kg) | 31.91±3.98 | 33.35±5.57 | 16.0 | 75.0 | 0.861 |
| SBP (mmHg) | 121.29±1.98 | 126.93±5.29 | 84.00 | 175.00 | 0.292 |
| DSP (mmHg) | 79.04±1.26 | 78.29±2.42 | 47.00 | 105.00 | 0.819 |
| Pulse (mg/dL) | 98.32±11.60 | 86.07±2.85 | 52.00 | 106.00 | 0.671 |

Suspected malaria patients

101 participants

15 Non-malaria patients

86 Malaria positive placed on microscopy

Anaemia blood on haemoglobin concentration

48

36

Non-Deficient G6PD 25

Deficient G6PD 11

Deficient G6PD 15 Haemoglobin Haematocrit MDA Protein Oxidation

Non-Deficient G6PD 33 Haematocrit

**Figure 1: Study flow tree for the recruitment of patients**

**Table 5: Relationship between anaemia and G6PD deficiency among malaria patient**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Characteristics |  | AnaemicPatients | Non-anaemic patients | *total* | *χ2* | *p-value* |
| Study participants | *Total* | 48 (57.1%) | 36 (42.9%) | 84 (100.0%) |  |  |
| G6PD | *Deficient* | 15 (17.9%) | 11 (13.1%) | 26 (31.0%) | *0.005* | 0.946 |
|  | *Non-deficient* | 33 (39.3%) | 25 (29.8%) | 58 (69.0%) |  |  |

**Table 6: Comparison of anaemia and oxidative stress indices among malaria patients**

|  |  |  |  |
| --- | --- | --- | --- |
| Characteristics (n =84) | Anaemicpatients | Non-anaemicpatients | *p-value* |
| Haemoglobin conc. (mg/dL) | 10.28±0.22 | 13.81±0.28 | < 0.001 |
| Haematocrit (mg/dL) | 30.23±0.65 | 40.47±0.85 | < 0.001 |
| MDA | 2.23±0.20 | 1.82±0.19 | 0.155 |
| Protein oxidation | 65.25±5.58 | 72.77±4.83 | 0.325 |

**Table 7: Interaction between anaemia and G6PD deficiency on study parameters**

|  |  |  |  |
| --- | --- | --- | --- |
| Characteristics (n =84) | Anaemicpatients | Non-anaemicpatients | 2 Way ANOVA *(p-value)* |
|  | **Deficient** | **Non-deficient** | **Deficient** | **Non-deficient** | **Anaemia status** | **G6PD status** | **Anaemia x G6PD** |
| Haemoglobin conc. (mg/dL) | 10.133±0.417 | 10.339±0.281 | 13.94±0.486 | 13.76±0.323 | <0.001 | 0.973 | 0.617 |
| Haematocrit (mg/dL) | 29.80±1.24 | 30.42±0.835 | 40.91±1.45 | 40.28±0.960 | <0.001 | 0.998 | 0.586 |
| MDA (µM) | 2.03±0.35 | 2.37±0.24 | 1.53±0.39 | 1.97±0.27 | 0.170 | 0.211 | 0.855 |
| Protein oxidation (µM) | 57.21±9.00 | 68.99±6.15 | 68.73±10.154 | 74.63±6.874 | 0.299 | 0.285 | 0.721 |

**CHAPTER FIVE**

**5.0 DISCUSSION, CONCLUSION AND RECOMMENDATION**

**5.1 DISCUSSION**

In total 101 patients were recruited for the study. After screening for malaria, 86 were found to be infected with malaria parasite while 15 patients were not infected with malaria parasite. Among, the 101 patients, 30 were male of which 24 were malaria patients while 6 male patients were not infected with malaria parasite. Among the 71 females, 62 were infected with malaria parasite while 9 were not infeceted with malaria parasite.

Comparison between RDT and microscopy techniques in diagnosing malaria showed RDT to have a poor performance in diagnosis malaria parasite compared to microscopy, the gold standard.

In the performance of RDT kits diagnosis of malaria using microscopy as standard, the performance showcased by RDT kit was low. In some cases, RDT kits displayed negative results when the test was carried out but when diagnosed with microscopy, a positive result was obtained showing the non-reliability of RDT in malaria diagnosis which may be of great detrimental effect to patients.

In the baseline characteristics study of participants, showed the statistical distribution of the malaria and non-malaria patients used in the study, there is no significant different using the *p* –value.

Anaemia is a condition that causes red blood cells to break down in response to certain medication, infections or other stresses, in the relationship between anaemia and G6PD deficiency among malaria patients in this study, the total number of anaemia patient was 48 while the non-anaemia patient was 36, the result showed that 15 anaemic patients were deficient while 11 non-anaemic patient were deficient. Also 33 anaemic patients were non-deficient while 25 non-anaemic patients were non-deficient.

Anaemia is a decrease in the total amount of red blood cells or haemoglobin in the blood resulting from a blood loss, decreased red blood cell production, it was observed in the comparison of anaemia and oxidative indices among malaria patients, that the haemoglobin concentration and haematocrit concentration were low in anaemia patient and high in non anaemia patients. It was also shown that in the detamination of protein oxidation, the MDA level was high in anaemia patient and low in non-anaemia patient.

These clearly suggests that G6PD deficiency is not associated to oxidative stress and malaria, rather it may be suggested that the production of G6PD may have other physiological effects apart from regenerating reduced glutathione. One of its functions which is to prevent haemoglobin oxidation and formation of methaemoglobin by converting Fe3+ to Fe2+ in the porphyrin ring of haemoglobin.

As revealed in this study, the methaemoglobin and haemoglobin were not significantly different in the malaria and non-malaria patients but is associated with G6PD deficiency, suggesting that G6PD deficiency promotes more methaemoglobin formation. Since Glucose-6-Phosphate Dehydrogenase (G6PD) is an enzyme found in the pentose phosphate pathway which maintains the level of coenzyme- Nicotinamide adenine dinucleotide phosphate (NADPH) (Takizawa *et al.,* 1986), which is required for methaemoglobin reductase activity and the maintenance of the level of reduced glutathione, regeneration of GSH and GSH-reductase requitrtes NADPH which is oxidized to NADP+ and reduced to gluthatione, in turn maintaining an effective redox potential that protects the cell membrane sulhydryl group, enzymes and oxidative stress.

**5.2 CONCLUSION**

Findings from this study showed that there was no relationship between, anaemia, G6PD deficiency and oxidative stress among malaria patients. However, malaria could influence haemoglobin and haemotocrit levels and hence lead to anaemia.

**5.3 RECOMMENDATION**

Since the sample size is small, it might be limiting to make general sample size. Hence, further study may be done with larger sample size and also since the study was conducted among Igbo population, further study may be needed to be conducted in other ethnic groups to ascertain this findings.

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**APPENDIX**

**Calculations:**

|  |  |  |  |
| --- | --- | --- | --- |
|  |  | Gold Standard(MICROSCOPY) |  |
|  |  | Positive | Negative |  |
| Test(RDT) | Positive | True Positive (TP) | False Positive (FP) | Total test positive |
|  | Negative | False Negative (FN) | True Negative (TN) | Total test negative |
|  |  | Total Disease | Total Non-Disease | Overall total (Sample size) |

Prevalence (%) = (Total Disease/Overall total) x100 or ((TP+FN)/Sample size) x100

Sensitivity = TP/(TP + FN) x100,

Specificity = TN/(TN + FP) x100,

Negative Predictive Value (NPV) = TN/(TN + FN) x100,

Positive Predictive Value (PPV) = TP/(TP + FP) x100

Accuracy = ((TP+ TN)/sample size) x100.