**THE RELATIONSHIP BETWEEN ANAEMIA, MALARIA AND ANTIOXIDANT STATUS IN PATIENT VISITING ENUGU STATE UNIVERSITY TEACHING HOSPITAL “ESUTH”**

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**GODFREY OKOYE UNIVERSITY,**

**UGWUOMU-NIKE, ENUGU STATE**

**JULY, 2018**

**THE RELATIONSHIP BETWEEN ANAEMIA, MALARIA AND ANTIOXSTATUS IN A PATIENTS VSITING ENUGU STATE UNIVERSITY TEACHING HOSPITAL, ENUGU.**

**PROJECT SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF A BACHELOR OF SCIENCE (B.Sc) DEGREE IN BIOCHEMISTRY**

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**JULY, 2018.**

**CERIFICATION**

 I Aniekwe Chioma Juliet an undergraduate student of the department of Chemical Sciences with registration number U15/NAS/BCH/034, 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 title “Relationship between anaemia, malaria and antioxidant status in patient visiting 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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**DEDICATION**

 I dedicate this work to the pillar of my life the Almighty God who has always been my source of inspiration, knowledge, wisdom, understanding and strength.

**ACKNOWLEDGEMENT**

I express my profound gratitude goes to the almighty God, the giver of grace and to my supervisor, Mr Engwa Azeh Godwill, for entrusting me with such work, and for the Efforts and been selfless in assisting me in every possible ways to ensure that this work is a success. I have learnt a lot working with you. I thank you for making me to go out of comfort zone which bring out the best in me.

I also sincerely acknowledge my H.O.D, Dr. Eugune Ayuk for the fatherly support, advice and direction he gave me. I also thank all the lecturers in the department for their understanding and encouragements within the period of my work.

My appreciation goes to my fellow students and colleague in the department, for their guidance and support in the course of this work.

My appreciation will never be complete if l don’t mention my beloved Late Mr Joseph and my honourable mum Mrs Janeth Nwachi Aniekwe and my lovely brothers and sisters for always fought tooth and nail to see that I am not in any sort of lack financially and their unending support morally and spiritually.

I cannot end this without appreciating a set of people who played significant roles during my project; Mr Arinze, Mr Frank etc.

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**Abstract**

Anemia is a condition that occurs when the amount of haemoglobin in a person’s drops below normal .and a decrease in haemoglobin is often associated with a decrease in the number of red blood cells and haematocrit which may be cause by malaria while Malaria is a major public health problem in Nigeria where it accounts for more cases and deaths than any other country in the world. Malaria is a risk for 97% of Nigeria’s population 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. (WHO, 2011). Malaria is a disease cause by a plasmodium parasite transmitted by the bite of infected mosquitoes. One of the key contributory factors to the development and progression of its complication is that malaria may develop anaemia deficiency and malaria can generate free radicals that can possibly promote the development of anaemia and oxidative stress; a condition characterized by increase production of free radicals or impaired antioxidant defense system. An antioxidant which help in the removal of free radicals thereby preventing oxidative stress. Hence, this study was aimed to investigate the relationship between malaria infection, anemia deficiency and antioxidant status of patients visiting ESUTH Enugu. The study was conducted at ESUTH Enugu, were the formal concept patients was obtained from patients. A total 101 patients were recruited for the study, a questionnaire was use to collected a basic characteristic of the patients after which a blood sample was collected to measure the haemoglobin conc, haematocrit conc, vitamin C, catalase, SOD( superioxide dimutase) of a patients were screened through various tests conducted in laboratory. According to the results obtained, 58 (57.4%) patients were anaemic while (43(42.6%) were non anaemic. The haemoglobin (10.33±0.194) **/ (**30.38±0.57), haematocrit (13.66±0.24)**/** (40.07±0.73) levelswere significant lower in anaemic patients than in the non anaemia patients (p- <0.001). While only the catalase showed a significant difference which elucidated that the catalase was higher in anaemic patents (1.45±0.03) compare to the non anaemia patients (1.35±0.03). Vitamin C and SOD conc level showed no significant between anaemic and non anaemic patients. There was no relationship between anaemia and malaria (p = 0.827). Also, there was no interaction between anaemia and malaria to influence the antioxidant indices as there was no significant difference among the anaemic and non-anaemic patients (p > 0.05). Findings from this study showed that there was no relationship between anaemia, malaria and antioxidant status of patient visiting ESUTH, Enugu.

**CHAPTER ONE**

1.0 **INTRODUCTION**

1.1 **Background of the Study**

Malaria is a common and life threatening disease in many tropical and subtropical areas and the term malaria originates from medieval Italian malaran: mala-aria “bad air”; the disease was formerly called *ague* or *marsh fever* due to its association with swamps and marshland. The term first appeared in the English literature about 1829. (Breeveld *et al*., 2012) There are currently over 100 countries and territories where there is high risk of malaria transmission.

 Malaria is one of the most important tropical infectious diseases. The annual worldwide incidence is estimated to be 300–500 each year with a mortality of between one and three million people. (Asante *et al*., 2004).

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), and Malaria is the 3rd leading cause of death for children under five years worldwide.

Malaria in Nigeria Malaria is a major public health problem in Nigeria where it accounts for more cases and deaths than any other country in the world. Malaria is at risk to 97% of Nigeria’s population. The remaining 3% of the population live in the malaria free highlands. (Kremsner *et* al., 200). There are an estimated 100 million malaria cases with over 300,000 deaths per year in Nigeria. This compares with 215,000 deaths per year in Nigeria from HIV/AIDS. Malaria contributes to an estimated 11% of maternal mortality. (WHO, 2012).

Malaria accounts for 60% of outpatient visits and 30% of hospitalizations among children under five years of age in Nigeria. Malaria has the greatest prevalence of 27.6 percent, in children age 6 to 59 months in the South East region. (Abubakar *et al*., 2002).

Malaria is a significant global problem. In 2015, there were 214 million cases of the disease worldwide, killing about 438,000 people. In other areas of the world, malaria causes substantial morbidity, especially in the rural areas of some countries in Asia and South America , and these countries are visited by more than 125 million international travelers every year and malaria is most often cause by travel to and from endemic areas. (Abubakar *et al*., 2002).

Malaria is a mosquito-borne infectious disease affecting humans and other animals caused by parasitic protozoan’s (a group of single-celled microorganisms) belonging to the Plasmodium types like *Plasmodium, falciparum, Plasmodium, vivax, Plasmodium, ovale, Plasmodium, malariae* and transmitted to humans/ animals by the bite of infected mosquitoes (WHO, 2014). The malaria parasite, entering the blood after an infective mosquito bite, which will infects/invades the red bloods cells. At the end of that infection cycle, red blood cell ruptures. The process lowers the amount of red blood cells and can cause anaemia which is low haemoglobin levels, frequently leading to anaemia. *Plasmodium facliparum* causes the most severe and profound anemia Malaria infection in human by plasmodium species is associated with a reduction with a significant risk of death, this cannot be explained simply by the direct destruction of parasitized red blood cells.

 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. (Becker K *et al.,* 2004). It was observed that erythrocytes infected with *Plasmodium. falciparum* produced OH• radicals and H2O2 about twice as much compared to normal erythrocytes. Higher level of these free radicals can lead to oxidative stress. (Becker K *et al*., 2004).

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 as well as by malaria parasite. The consequence of oxidative stress once it is high, it can attack cellular membrane lipids, causing damage to cells and tissues such as the brain, metabolic disorders or inherited disease affecting electron transport chain.

Oxidative stress during malaria is considered useful to the patient in the fight against the intra-erythrocytic parasite (Gilbert D.L. 1981). Studies have been described in which induction of oxidative stress by treatment with pro-oxidants proved to be effective against the infection. On the other hand ROS play a role in the pathology of malaria (Jayshree et al., 1993). Excessive oxidative stress particularly at unprescribed sited (e.g. vascular lining, blood brain barrier) can damage the defense system. This is however, controlled by intra- and extracellular anti-oxidants systems, which may fail during disease. Treatment with anti-oxidants reinforces these systems and protects the patient, especially during the life threatening phase of the disease (Rice-Evans et al., 1992).

In malaria infections, the most probable target of free radical generated by malaria parasite is the red blood cell since it is where the parasite resides. Thus, though malaria parasite infection also damage the red blood cell during its asexual stage multiplication, free radicals produced by the parasite may also contribute. Thus, it may be suggested that anaeamia which is defined by the reduction of haemoglobin in blood below normal may result from the parasite damaging the red blood cell as well as free radical generation Anemia is a condition that occurs when the amount of haemoglobin in a person’s drops below normal .and a decrease in haemoglobin is often associated with a decrease in the number of red blood cells and haematocrit. Haemoglobin is contained with RBCs and it is necessary to transport and delivery of oxygen from the lungs to the rest of the body. Without a sufficient supply of oxygen, many tissues and organs throughout the body can be adversely affected. People with anemia may experience faigue weakness / tiredness, and may lack energy, breathlessness. groups , However, certain people have increase risk of developing anemia Anaemia fairly common condition affecting both men and women of all ages, race and ethnic. These include people with diets poor in irons and vitamins, chronic diseases such as kidney disease and inflammatory bowel disease. Therefore, increased level of anaemia in malaria may be due to the influence of free radical induced oxidative stress.

Antioxidants are molecules that inhibit or quench free radical reactions and delay or inhibit cellular damage (Adam-Vizi, V. (2005). 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 (Adam-Vizi, V. (2005). The major enzymatic antioxidants are superoxide dismutase, catalyze and glutathione peroxidase. Superoxide dismutase exists as a copper, zinc-enzyme (SOD1) that is found in the cytoplasm or a manganese containing enzyme that is located in mitochondria (SOD2). These enzymes catalyze the one-electron dismutation of superoxide (O2·) to hydrogen peroxide (2O2 + 2H+ → H2O2 + O2). Catalyse is an iron-dependent enzyme that directly decomposes hydrogen peroxide to water (2H2O2 → 2H2O + O2). Furthermore, glutathione peroxidases (GPXs) are a family of enzymes that reduce a variety of organic and inorganic hydroperoxides to the corresponding hydroxyl derivatives in the presence of glutathione (GSH). In this process, GSH is converted to an oxidized disulfide (2GSH + H2O2 → GS-SG + 2H2O).

The other way of categorizing the antioxidants is based on their solubility in the water or lipids. The antioxidants can be categorized as water-soluble antioxidants. The water-soluble antioxidants (e.g. vitamin C) are present in the cellular fluids such as cytosol, or cytoplasmic matrix.

1.2 **Statement of the problem**

Malaria remains one of the leading causes of morbidity and mortality worldwide and in sub- Saharan Africa. (WHO, 2012). Mortality from malaria is due to complication arising as a result of severe infections usually caused by *Plasmoduim Falciparum*. Studies on mortality have shown that deaths occur predominantly among young children/ some adult and mortality rates among patients with an illness severe enough to warrant hospitalization are consistently high with case fatality rates varying from 5% to 30% in Nigeria, malaria is hyper endemic and presents a serious health problem in the country. It is also a leading cause of deaths in the country and accounts for over 40% of out-patient attendance with annual reported cases and children less than five years are the most affected. A study conducted by Ministry of Health in 2006 showed that more than 17 million of Nigerian’s over twenty million people are infected with malaria/anaemia every year, with cost of $95 million for treatment. Despite the importance of *Plasmoduim Falciparum*. As a human pathogen, the patho-phyrsiologic basis of its infection is not well understood. Parasitic infections such as malaria in host organisms often lead to anaemia and oxidative stress condition which is a disturbance in the balance between the production of ROS and antioxidant defenses .generation of free radicals as a result of oxidative stress and other reactive species in vivo leads to extensive damage in parasite bio- molecules such as DNA, lipids and proteins. It has also been shown that the parasites are vulnerable to oxidative stress during their erythrocytic life stages. In erythrocytes, *Plasmoduim Falciparum*. Encounters enhanced oxidative stress, resulting largely from its digestion of haemoglobin and thus, its redox balance becomes fragile. Superoxide (O2-) is normally produced when oxidized haemoglobin is exposed to the acid environment of the food vacuole, and can therefore be considered as the major source of ROS. Inside the parasite, regardless of its origin, O2- is dismutated by superoxide dismutase (SOD) to H2O2 (Makani, J*. et al*., 2010). Even though studies have been carried out on *Plasmoduim Falciparum* and ROS, the focus has mainly been on the pathological effect of these radicals.

1.3 **Hypothesis**

Malaria may promote oxidative stress and anaemia and reduce antioxidants.

1.4 **Aim of the study**

The study was undertaken to evaluate the relationship between anaemia, malaria infection, and antioxidant status of patients visiting Enugu State University Teaching Hospital (ESUTH) in Enugu Nigeria.

1.5 **Objective of the study**

1. To determine the presence of malaria parasite infection in patients
2. Determine haemoglobin concentration and haemoglobin to assess anaemia in infected patients.
3. Determine antioxidant indices (SOD, and Vitamin C) among malaria patients.

**CHAPTER TWO**

 2.0 **LITERATURE REVIEW**

2.1 **History of Malaria and Associated Anaemia**

Anemia is the most common blood disorder, affecting about a third of the global population. [Iron-deficiency anemia](https://en.wikipedia.org/wiki/Iron-deficiency_anemia) affects nearly 1 billion people. In 2013, anemia due to iron deficiency resulted in about 183,000 deaths – down from 213,000 deaths in 1990. It is more common in women than men, during pregnancy, and in children and the elderly. Anemia increases costs of medical care and lowers a person's productivity through a decreased ability to work. the name was derived from the ancient Greek word called anaimia, meaning lack of blood.

(<https://en.m.wikipedia.org/wiki/anemia>)

Malaria is one of the oldest diseases believed to have infected man over 50,000 years (<http://en.wikipedia.org/wiki/Malaria>). And the evidence of this was first found in the Xian Dynasty and Medieval Europe through archeological studies. The name originated from the Italian word malaria meaning ―bad air‘ and it is believed to have influenced to a great extent of human history. Although the parasite was first noticed in the red blood cell in 1880, by Charles Louis Alphonse Laveran, a French Medical surgeon and proposed that it was a protozoan disease, it was not until 1886 that another scientist, Camillo Golgi, an Italian neurologist established that the parasite has at least two disease forms, the tertian periodicity (fever every other day) and the quartan periodicity (fever every third day). (<http://en.wikipedia.org/wiki/Malaria>) He also observed that these forms produced differing numbers of merozoites when they matured and fever always occurred with the release of the merozoites into blood circulation. In 1890, two other scientists, Giovanni Batista Grassi and Raimondo Filetti assigned the names Plasmodium vivax and Plasmodium malariae for two of the malaria parasites affecting humans. Also, in 1897, William H. Welch, an American, named Plasmodium falciparum as the parasite responsible for the malignant tertian form. In 1922, the fourth human malaria parasite was described by John William Watson Stephens. In 1897, Ronald Ross established that the malaria parasite was transmitted by infected mosquitoes. He did this by isolating the malaria parasite from the salivary glands of mosquitoes that bit malaria infected birds and then transmitted the parasite to healthy birds. This led to the discovery of the transmission of the human malaria parasite Plasmodium between 1898- 1899 by a team of Italian investigators led by Giovanni Batista Grassi

**2.2 Global View of Malaria**

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Figure1

Global spatial distribution of Plasmodium falciparum and global distribution of Plasmodium vivax malaria (source: MAP. Malaria atlas project. http://www.map.ox.ac.uk.

Malaria remains an important infectious disease in tropical and subtropical regions, and a major global health problem, with over 40% of the world`s population exposed to varying degrees of risk of this infection in over 100 countries. (http//www.mmv.org.) It is estimated that over 500 million people suffer from malaria infection annually, resulting in about 1-2 million deaths, of whom 90% are children in sub-Saharan Africa ((http//www.mmv.org.)). Beside Sub- Saharan Africa, other developing countries most affected are South and South-eastern Asia, Oceania and Haiti, where *Plasmodium. falciparum* malaria prevails. A critical feature that may help you to recognise if a fever is due to malaria or not is that **malarial fever** occurs in cycles – periods of fever alternate with periods in which the patient shows normal body temperature (below 37.5°C) and no symptoms. (Makani *et al*., 2010).



Fig. 2

Ring-forms and gametocytes of *Plasmodium falciparum* in human blood.

Source: [https://en.wikipedia.org/wiki/file:plasmodium.jpg](https://en.wikipedia.org/wiki/file%3Aplasmodium.jpg)

**2.3 Anemia**

 Anemia is a condition that occurs when the amount of hemoglobin in a person’s drops below normal. a decrease in hemoglobin is often associated with a decrease in the number of red blood cells and haemtocrit. Anamia (from Greek word *Anaimia,* meaning lack of blood. It is a decrease in number of red blood cells (RBCs) or less than the normal quantity of heamoglobin in the blood. Hemoglobin is contained with RBCs and it is necessary to transport and delivery of oxygen from the lungs to the rest of the body. Without a sufficient supply of oxygen , many tissues and organs throughout the body can be adversely affected. (Makani *et al*., 2010). People with anemia may experience fatigue, weakness/tiredness, and may lack energy, breathlessness.

Anaemia fairly common condition affecting both men and women of all ages, race and ethnic groups. However, certain people have increase risk of developing anemia. These include people with diets poor in irons and vitamins, chronic diseases such as kidney disease, diabetes, cancer and inflammatory bowel disease.

**2.4 Causes of anemia**

Impaired or decrease production of red blood cells by the bone marrow due to nutritional status like antioxidant (example ascorbic acid ), bone marrow failure (e.g anaemia) (Makani *et al.,* 2010). Loss of red blood cells due to bleeding or increase destruction of red blood cells as anaemia. Anaemia can be chronic and acute **Anaemia** means not enough haemoglobin in the blood. Haemoglobin is the red substance in the red blood cells which carries oxygen. Malaria parasites destroy the red blood cells and so malaria may cause anaemia. Anaemia may also have other causes (for example, not enough iron in the food). You can recognise anaemia by looking at the patient’s hands: the palms of a person with anaemia do not have the redness of a healthy person’s palms. If the red colour of the inner eyelid or mouth is paler than in a healthy person, the patient has anaemia. Breathlessness and a fast pulse may also be present, because the person’s blood cannot carry enough oxygen for their needs. Infections like [malaria](https://en.wikipedia.org/wiki/Malaria), and certain autoimmune diseases.can also be classified based on the [size of red blood cells](https://en.wikipedia.org/wiki/Mean_corpuscular_volume) and [amount of haemoglobin in each cell](https://en.wikipedia.org/wiki/Mean_corpuscular_hemoglobin). If the cells are small, it is [microcytic anaemia](https://en.wikipedia.org/wiki/Microcytic_anemia). If they are large, it is [macrocytic anaemia](https://en.wikipedia.org/wiki/Macrocytic_anemia) while if they are normal sized, it is [normocytic anaemia](https://en.wikipedia.org/wiki/Normocytic_anemia). Diagnosis in men is based on a haemoglobin of less than 130 to 140 g/L (13 to 14 g/dL), while in women; it must be less than 120 to 130 g/L (12 to 13 g/dL). Further testing is then required to determine the cause.

Certain groups of individuals, such as pregnant women, benefit from the use of [iron pills](https://en.wikipedia.org/wiki/Iron_pill) for prevention. [Dietary supplementation](https://en.wikipedia.org/wiki/Dietary_supplements), without determining the specific cause, is not recomm ended. The use of [blood transfusions](https://en.wikipedia.org/wiki/Blood_transfusion) is typically based on a person's signs and symptoms. In those without symptoms, they are not recommended unless haemoglobin levels are less than 60 to 80 g/L (6 to 8 g/dL). These recommendations may also apply to some people with acute bleeding. [Erythropoiesis-stimulating medications](https://en.wikipedia.org/wiki/Erythropoiesis-stimulating_medications) are only recommended in those with severe anaemia.

**Table 1: WHO’S Hemoglobin threshold used in to define Anaemia (1g/dl = 0.6206 mmol/L)**

|  |  |  |
| --- | --- | --- |
| **Age or gender group** | **Hb threshold (gl/dl)** | **Hb threshold (mmol/l)** |
| Children (0.5-50 yrs) | 11 | 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 |

Source: <https://en.m.wikipedia.org/wiki/anemia> )

2.5  **A etiology of Malaria**

 Malaria is a mosquito-borne infectious disease affecting humans and other animals caused by parasitic protozoan’s (a group of single-celled microorganisms) belonging to the *Plasmodium* types like *Plasmodium falciparum,* *Plasmodium* *vivax*, *Plasmodium* *ovale*, *Plasmodium* *malariae* and transmitted to humans/ animals by the bite of infected mosquitoes (WHO, 2014). The malaria parasite, entering the blood after an infective mosquito bite, which will infects/invades the red bloods cells. At the end of that infection cycle, red blood cell ruptures. The process lowers the amount of red blood cells and can cause anemia which is Low hemoglobin levels, frequently leading to anemia. *Plasmodium facliparum* causes the most severe and profound anemia Malaria infection in human by plasmodium species is associated with a reduction in with a significant risk of death, this cannot be explained simply by the direct destruction of parasitized red blood cells.

**2.6 The Life Cycle of the Malaria Parasite**

The Plasmodia parasite has a complex, multistage life cycle which involves an insect vector (the mosquito) and the vertebrate host (human). The presence of more than 5,000 parasite genes and their specialized proteins aid the parasite to invade and develop within multiple cell types and to evade host immune responses thus, ensuring the parasite survival and development inside the invertebrate and vertebrate hosts, in intracellular and extracellular environments. (Greenwood **et** *al.,*2008). Four species are known to infect man: *Plasmoduim falciparum*, *Plasmoduim*i, *Plasmoduim* *ovale* and *Plasmoduim* *malariae;* all of which exhibit a similar life cycle with only minor variations. The parasite goes through various phases during its developmental cycles such as the sporozoites, merozoites, trophozoites, (asexual schizogony stage) and gametocytes (sexual sporogony stage) and all of these stages have been found to have their unique shapes and structures as well as protein complements. As the parasite goes through the different stages of its cycle the surface proteins and metabolic pathways change, making it possible for the parasite to elude the immune clearance, thereby creating problems for the development of drugs and vaccines (Laurence et al., 2002). The Asexual Schizogony Phase Man is the intermediate host for malaria, in which the asexual phase of the life cycle occurs. This phase of the cycle is initiated from the liver when the infested anopheles mosquito inoculates the sporozoites during a blood meal, and the latter part continues inside the red blood cells, which results in the various clinical manifestations of the disease.



Fig.3 life cycle of malaria parasite

Source: (wiki images)

A mosquito causes an infection by a bite. First, sporozoites enter the bloodstream, and migrate to the liver. They infect [liver cells](https://en.wikipedia.org/wiki/Liver_cells), where they multiply into merozoites, rupture the liver cells, and return to the bloodstream. The merozoites infect red blood cells, where they develop into ring forms, trophozoites and schizonts that in turn produce further merozoites. [Sexual forms](https://en.wikipedia.org/wiki/Gametocytes) are also produced, which, if taken up by a mosquito, will infect the insect and continue the life cycle.

**2.6.1 Phases of Malaria**

**The Asexual Schizogony Phase**

Man is the intermediate host for malaria, in which the asexual phase of the life cycle occurs. This phase of the cycle is initiated from the liver when the infested anopheles Mosquito inoculates the sporozoites during a blood meal, and the latter part continues inside the red blood cells, which results in the various clinical manifestations of the disease.

**2.6.2 The Pre-erythrocytic Stage**

During blood meal by the infested mosquito, hundreds of sporozoites are introduced into the intradermis. Some of these sporozoites are destroyed by the local macrophages, while others find a blood vessel. Some of the surviving sporozoites enter the lymphatic vessel into the draining lymph node where some of them partially develop into exoerythrocytic stages and may also activate the T cells to build up a protective immune response. (Vaughan *et al*., 2008).

The sporozoites that enter the blood vessel invade the liver within a few hours. Recent studies have shown that the sporozoites travel by a continuous sequence of stick-and-slip motility, using the thrombospondin-related anonymous protein (TRAP) family and an actin–myosin motor. *P. knowlesi* Inside the liver the sporozoites develop into schizonts, each containing 10,000–30,000 merozoites or more in case of *Plasmoduim falciparum*. (Vaughan et al, 2008).

In the liver, the parasite growth and development is made easier by a condusive environment created by the circumsporozoite protein of the parasite. The whole pre-erythrocytic phase lasts about 5–16 days depending on the parasite species on an average 5-6 days for *P. falciparum*, 8 days for *Plasmodium vivax*, 9 days for *Plasmoduim ovale*, 13 days for *Plasmoduim malariae* and 8-9 days for at maturity, the merozoites are released into the blood stream and invade red cells by multiple receptor–ligand interactions.

In P. vivax and P. ovale malaria, some of the sporozoites remain dormant for months within the liver. These are called hypnozoites, and develop into schizonts after some latent period, usually of a few weeks to months. It is suspected that these hypnozoites are genotypically different from the sporozoites that cause acute infection soon after the inoculation by a mosquito bite , and in some patients cause relapses of the clinical infection after weeks to months. (Vaughan *et al.,* 2008).

**2.6.3 Erythrocytic Stage**

The asexual development of the malaria parasite occurs in the red blood cells. It is suspected that the disappearance of the parasite from circulation into the red cells reduces the exposure of its surface antigens, thus protecting it from the host immune response. (Greenwood *et al*., 2008). Interaction between the parasite and the red cell causes alteration across the red cell membrane, resulting in the formation of a stable parasite–host cell junction. As a result, the parasite enters the erythrocyte with the aid of the actin–myosin motor, proteins of the thrombospondin-related anonymous protein family (TRAP) and aldolase, forming a parasitophorous vacuole to seal itself from the host-cell cytoplasm, thus creating a hospitable environment for its development in the red cell. The parasite at this stage appears as an intracellular ring.

Within the red cells, the parasite multiplies rapidly. The parasite ingests the haemoglobin in the red cell into a food vacuole and degrades it. It utilizes the amino acids in the haemoglobin for protein biosynthesis and the heme is detoxified by heme polymerase and sequestrated as hemozoin (malaria pigment). The malaria parasite also depends on anaerobic glycolysis for energy, utilizing enzymes such as pLDH, plasmodium aldolase etc.. As the cycle progresses, the merozoites develop and divide within the vacuole each into fresh merozoites, trophozoites, and schizonts. Some of the merozoites at this stage do not undergo schizogony but differentiate into the sexual stage male and female gametocytes. These forms are extracellular and nonpathogenic and help in transmission of the infection to others through the female anopheline mosquitoes, inside which they continue the sexual phase of the parasite's life cycle. (Silvie et al, 2008).

**2.6.4 Sexual Sporogony Phase**

The sexual phase of the parasite's life cycle occurs in the mosquito which is the definitive hosts. The sexual phase results in the development of infecting forms of the parasite within the mosquito that stimulate disease in the human host following their injection during blood meal. During a blood meal the female Anopheles mosquito picks up the male and female gametocytes of the parasite which find their way into the gut of the mosquito where they develop into the gamete forms. The male and female gametes fuse in the mosquito gut to form zygotes, which subsequently develop into actively moving ookinetes that moves into the mosquito‘s midgut wall to develop into oocysts. These further develop and divide into active haploid forms called sporozoites which find their way into the body cavity of the mosquito, from where they travel to and invade the mosquito salivary glands. When the mosquito at this stage takes another blood meal, the sporozoites get injected from its salivary glands into the human bloodstream, causing malaria infection in the human host. (Barillas-Mary and Kumar, 2005).

**2.7 Transmission of Malaria Parasite**

The disease is most commonly transmitted by infected female Anopheles mosquitoes, which bite between dusk and dawn. The mosquito bite introduces the parasites from the mosquito's saliva into a person's bloodstream (WHO, 2014). Once the parasites are inside one’s body, they travel to the liver, where they mature and reproduced. After several days, the matured parasites enter the bloodstream and begin to infect the red blood cells. Five species *Plasmodium* can infect and be spread by humans. (Ferguson and Read, 2004). Most deaths are caused by *Plasmodium falciparum.*

**2.8 Complications Of Malaria/ Anameia, Oxidative Stress and Antioxidan**

 One can get malaria if you’re bitten by an infected mosquito or if you receive infected blood from someone during a blood transfusion, malaria can also be transmitted from mother to fetus during pregnancy.

The mosquitoes that carry plasmodium parasites get it from biting a person or animal that’s already been infected. The parasite then goes through various changes that enable it to infect the next creature the mosquito bites. Once it’s in you, it multiplies in the liver and changes again getting ready to infect the next mosquito that bites you. It then enter bloodstream and invades red blood cells. Eventually, the infected red blood cells burst. This sends the parasites throughout the body and causes symptoms of malaria. (Ferguson and Read, 2004).

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. (Becker *et al*, 2004). It was observed that erythrocytes infected with *Plasmodium. falciparum* produced OH• radicals and H2O2 about twice as much compared to normal erythrocytes. Higher level of these free radicals can lead to oxidative stress. (Becker *et al*, 2004).

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 as well as by malaria parasite. The consequence of oxidative stress once it is high, it can attack cellular membrane lipids, causing damage to cells and tissues such as the brain, metabolic disorders or inherited disease affecting electron transport chain.

Oxidative stress during malaria is considered useful to the patient in the fight against the intra-erythrocytic parasite (Gilbert, 1981). Studies have been described in which induction of oxidative stress by treatment with pro-oxidants proved to be effective against the infection. On the other hand ROS play a role in the pathology of malaria (Jayshree *et al*., 1993). Excessive oxidative stress particularly at unprescribed sited (e.g. vascular lining, blood brain barrier) can damage the defense system. This is however, controlled by intra- and extracellular anti-oxidants systems, which may fail during disease. Treatment with anti-oxidants reinforces these systems and protects the patient, especially during the life threatening phase of the disease (Rice-Evans *et al*., 1992).

In malaria infections, the most probable target of free radical generated by malaria parasite is the red blood cell since it is where the parasite resides. Thus, though malaria parasite infection also damage the red blood cell during its asexual stage multiplication, free radicals produced by the parasite may also contribute. Thus, it may be suggested that aneamia which is defined by the reduction of haemoglobin in blood below normal may result from the parasite damaging the red blood cell as well as free radical generation. Therefore, increased level of anaemia in malaria may be due to the influence of free radical induced oxidative stress.

**2.9 Factors that determine the occurrence of malaria**

 For malaria to occur in any environment, three components must be present: humans, anopheles mosquitoes, and parasites. Anopheles mosquitoes must be in contact with humans, and the parasites must be in contact with humans to complete the "invertebrate host" half of their life cycle. However, in rare occasions, malaria parasites can be transmitted from one person to another without requiring passage through a mosquito, as in malaria transmission from mother to child (congenital malaria), shared needles, [blood transfusion](https://www.omicsonline.org/bone-marrow-research.php), and organ transplantation.

**2.9.1 Climate**

 All three components can be influence by a number of things, the most important of which is the climate. Changing climate conditions give rise to increased infectious disease such as malaria. Water from rainfall can accumulate in places that become breeding sites for anopheles mosquitoes to lay and deposit their eggs, and larvae and pupae develop into adulthood. This process takes approximately 9-12 days in most tropical countries. The surrounding temperature, rainfall, and humidity determine the survival chances of these adult mosquitoes. For malaria transmission to be successful, female anopheles must survive long after they infect the human blood to enable the parasites to complete their growth cycle. These cycles range anywhere between 9 to 21 days with an ambient temperature that ranges between 25°C or 77°F. The warmer the temperature, the shorter the cycle, a condition that elevates the chances of transmission. Climate also determines human behaviors that may increase contact with Anopheles mosquitoes between dusk and dawn, when the anopheles are most active. Hot climate conditions increase the chances of people contracting food poisoning, malaria, and a host of other infectious diseases. Hot weather may encourage people to sleep outdoors or discourage them from using bed nets exposing themselves mosquito bites. During harvest seasons, agricultural workers might sleep in fields or nearby locales, without protection against mosquito bites. It has been speculated that current trends of global warming may increase the geographic range of malaria and may be responsible for malaria epidemic. Global environmental degradation that includes soil erosion from heavy rains, deforestation, no clean drinking water and clean environments all contribute to increased risks of infectious disease including malaria.

### 2.10 Signs and Symptoms of Malaria

Symptoms of malaria usually start to appear 7 to 21 days after the bite of an infected mosquito. However, the normal incubation period is different for different species of Plasmodium, as described in Study Session 5. Remember that the **incubation period** is the time between the parasite getting into the blood of a person and the onset of symptoms. (WHO, 2010).

The clinical symptoms of malaria vary from very mild to very severe, depending on several factors. In areas where malaria is very common, adults with the disease may show just a slight increase in body temperature. However, pregnant women, and in particular, young children, often have a severe illness with many symptoms. The most important symptom of malaria is fever (or a history of fever within the last two to three days). An attack often begins with shivering (body shaking). This is followed by a period of fever, and finally there is profuse sweating. During an attack the patient often complains of headache and pains in the back, joints, and all over the body.

There may also be loss of appetite, vomiting, and diarrhoea. The patient may feel better the next day, but may have another attack the day after that, and so on. If untreated (or inadequately treated), malaria can cause several weeks or months of poor health because of repeated attacks of fever, **anaemia** (see table 1) and general weakness. Some patients rapidly become very ill and may die within a few days. (Kremsner *et al*., 2000).

**Table 2 Clinical symptoms of a typical malarial fever attack.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|

| **Stage of malarial fever attack** |
| --- |
| **Stage name** |

 | **Cold stage** | **Hot stage** | **Sweating stage** |
| Main clinical symptoms | Feeling very cold Vigorous shivering | Feeling very hot – higher than normal temperatureDry burning skinHeadache  | A lot sweatingFall in temperature Feeling exhausted and weakTendency to fall asleep |
| How long symptom last | 15 - 60 minutes  | 2 - 6 hours | 2 - 4 hours |

(Kremsner et al., 2000)

**2.11** **Malaria Diagnosis**

If you suspect that a patient may have malaria, you will need to confirm the clinical diagnosis using specific tests to identify the presence of the malaria parasite or its products in the blood. This process is called **parasitological or parasite-based diagnosis.** In areas with a risk of malaria, or in patients who have travelled back from malaria-endemic areas, fever should be enough to make you suspect malaria and do a confirmatory test. The parasitological diagnosis of malaria can be divided into microscopic and non-microscopic tests. Microscopic tests involve the use of a microscope to see the parasite in the blood of a patient. (Vaughan *et al*., 2008).

### 2.12 Rapid Diagnostic Test (RDT)

RDTs test whether a person with malaria-like symptoms actually has malaria by testing the blood of the patient for chemical substances produced by malaria parasites. Malaria parasites produce proteins called antigens. RDTs detect malaria antigens, so if they are present, the person will test positive. If malaria antigens are not present, the person will test negative. (Vaughan et al, 2008).

### The reason for using RDTs

RDTs enable you to find out if a fever is really caused by malaria rather than by other illnesses. You can also get information about which malaria parasites may be causing the infection. The information provided by RDTs is important for three main reasons:

* First, being able to tell quickly whether a patient with fever has malaria or not ensures that the patient can receive the correct treatment.
* Second, if a patient does have malaria, knowing which parasite may be involved is important, as some malaria parasites are more dangerous than others and require more urgent treatment.

**2.12.1 Microscopic Examination**

Thick smear: minimum of 200 fields was examined for the presence of blood parasites under oil immersion objective. Thin smears: minimum of 200 fields under oil immersion objective was examined for species identification.

**2.13 Malaria Disease and Oxidative Stress**

The role of oxidative stress during malaria infection is still unclear; whiles some school of thoughts suggest a protective role, others claim a relation to the physiopathology of the disease . However, recent studies have suggested that reactive oxygen and nitrogen species (ROS and RNS) associated with oxidative stress play an important role in the development of systemic complications caused by malaria. Malaria infection induce hydroxyl radicals (OH•) generation in the liver, which may probably be the main reason for the induction of oxidative stress and apoptosis. Furthermore, (Hunt and Stocker, 1990), observed that erythrocytes infected with *Plasmodium falciparum* produced OH• radicals and H2O2 twice as much compared to normal erythrocytes. The host‘s haemoglobin molecule is a possible source of free radical production in this disease, since the parasite utilizes it for its amino acid needs for its own nutrition during the erythrocytic stage of the disease, resulting in the release of large amounts of circulating haem. These haems have Fe2+ associated groups which are able to induce intravascular oxidative stress, resulting in changes in erythrocytes and endothelial cells, thus, facilitating the internalization of the parasite in tissues such as the liver and brain. A free radical species, which appears to be involved in this disease, is nitric oxide. However, its role is still controversial. Oxygen radicals have been shown to be important for the clearance of disease in mice and humans. This therefore, suggests that reduced production of ROS by monocytes might aggravate infection and may contribute to the disease manifestation.

 **2.14** **Oxidative Stress in Plasmodium Falciparum Infected Erythrocytes**

Ring-forms and [gametocytes](https://en.wikipedia.org/wiki/Gametocyte) of *Plasmodium falciparum* in human blood.

Malaria parasites are particularly vulnerable to oxidative stress during their erythrocytic life stages. The parasites live in a pro-oxidant environment that contains oxygen and iron which are the key requirements for the formation of reactive oxygen species (ROS). The parasites take up haemoglobin into their acid food vacuole which leads to the oxidation of Fe2+ to Fe3+ and the formation of superoxide anions. This combination leads to the generation of hydrogen peroxide and subsequently hydroxyl radicals, both highly reactive and toxic oxygen intermediates. (Laurence et al., 2002). Apart from the parasite itself being under oxidative stress, the host cell also comes under oxidative alterations when infected with Plasmodium. Changes in erythrocyte membrane fluidity, most probably because of alterations of erythrocyte membrane lipid compositi reactive and toxic oxygen intermediates. Furthermore, toxic haem (ferri/ ferroprotoporphyrin IX; FP IX) is released upon haemoglobin digestion and this must be detoxified. Most of the released FP IX is biomineralized (up to 90%); to form inert haemozoin. It is suggested that an appreciable amount of FP IX (even as much as 50%); (Laurence et al., 2002). escapes biomineralization and is degraded or sequestered by other means to prevent membrane damage and parasite death on and protein cross linking suggest that. Oxidative stress is commonly observed to arise from five sources during disease physiopathogeny:

1. inflammatory process initiated in the host in response to infection;
2. Transition metal catalysis, since in feeding on hemoglobin, the parasite releases significant amounts of free iron;
3. The occurrence of ischemia-reperfusion syndrome, resulting from cytoadherence processes and anemia triggered by infection.
4. Direct reactive species production by the parasite; and
5. Action of anti-malarial drugs.

Oxidative stress has a protective role in malaria patients as possible agents capable of destroying the Plasmodium. Thus, H2O2 and O2•− can operate independently as cytotoxic agents or form other toxic molecules, including radical OH•, hypochlorous acid (HOCl) and peroxynitrite (ONOO−) in the presence of NO ROS generated by macrophages are non-specific effectors molecules in the host‘s defense arsenal, which can contribute to oxidative damage in the parasite as well as parasitized erythrocytes, once ROS are able to diffuse through the membrane of red blood cells.

Also, neutrophils secrete proteolytic enzymes and ROS, which in low concentrations can trigger apoptosis of endothelial cells and necrosis in high concentrations. Plasmodium falciparum trophozoites increase the viscosity of red blood cells by causing changes in the parasitized cell surface thus, permitting its adhesion to the endothelial wall of capillaries, which seems to be a defense mechanism of the parasite, thereby preventing the passage of parasitized red blood cells through the spleen and their consequent destruction. 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. Lipid peroxidation occurs on the surface of the infected red blood cells. The parasitized erythrocytes contain large amounts of monohydroxy derivatives of polyenoic fatty acids (OH-PUFA) in their lipids, which suggest that the episode of lipid peroxidation is as a result of the release of haem iron from non-enzymatic breakdown . One of the common OH-PUFA (12- and 15-hydroxy-arachidonic acid (HETE)) increases according to the evolutionary stage of the parasite. Low concentrations are found after phagocytosis of parasitized RBCs, suggesting that other lipid peroxidation products also may play a key role in this process. Additionally, accelerated aging of these cells is attributed to oxidative changes in P. falciparum-infected red blood cells and is reported to contribute to the development of anemia. This promotes changes in the circulatory physiology, which results in moments of hypoxia alternating with the maintenance of tissue oxygenation at basal levels, favoring the participation of is chemicals and reperfusion syndrome (IRS) accountable for additional free radicals production.

2.15 **Oxidative Changes in Plasmodium**

In addition to the host producing ROS/RNS during infection, the parasite is also capable of producing free radicals, which in turn hinder the biochemistry of red blood cells and may facilitate the internalization of the parasite in hepatocytes and RBC. Plasmodium parasites are exposed to high levels of oxidative stress during development in host cells. Their ability to protect themselves against this hostility is important to their continued existence. As a result, these parasites have developed several antioxidant defense mechanisms. Gene expression during the erythrocytic phase of infection by Plasmodium suggests there is a continuous cascade of gene expression at the early stages, and five different proteins with antioxidant properties are expressed. Additionally, Plasmodium reduces its own production of reactive oxygen species and adapts new mechanisms to prevent oxidative damage arising from the host. This, the parasite does to compensate for the oxidative stress suffered. One such mechanisms is the apicoplast; it is a symbiotic intracellular organelle located near the mitochondria which seems to synthesize lipoic acid, a potent antioxidant the parasite uses as a defense. (Laurence *et al*., 2002).

**2.16 Oxidative Changes in The Host Induced By Plasmodium**

During malaria infection, the host natural defense machinery is activated with involvement of phagocytes (macrophages and neutrophils). These, sequentially, produce huge amounts of ROS and RNS, resulting in an imbalance between oxidizing species formation and antioxidants activity. This imbalance triggers oxidative stress, which is an important machinery of human hosts in response to infections and can lead to the death of the parasites. The ability of oxidative stress to promote the killing of parasites has been established in in vitro studies. Plasmodium yoelii species grown in the presence of glucose and glucose oxidase generated H2O2, a reactive oxygen species, capable of killing the parasite; free radical superoxide (O2•−) when grown in the presence of xanthine and xanthine oxidase, and an ensuing burst of further oxidative products, with subsequent damage to the parasites. Moreover, oxidative stress indicators are found to be high in infected humans. This result from increased free radicals production, suggested by increased malondialdehyde (MDA) which is an important lipid peroxidation marker, signifying that oxidative stress is important machinery in parasite infection. Oxidative stress according to studies can take part in the pathogenesis of thrombocytopenia associated to malaria. It has been suggested that Plasmodium vivax malaria infected individuals have reduced platelets number and antioxidant enzymes—superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities while lipid peroxidation of platelets (estimated by measuring the MDA), is elevated. Beside the synthesis of radical species, organisms have developed several antioxidant defense mechanisms in response to increased oxidative stress. Antioxidant defense is an innate physiological mechanism of organisms against damage caused by free radicals and is dependent on the utilization of cellular and systemic antioxidant reserves. Endogenous synthesis of these antioxidant compounds usually consists of three interdependent systems: enzymatic, small molecules and metal chelating, which prevent oxidation of bimolecular. The antioxidant defense system also halts oxidative species generation by scavenging or by free radicals reduction, which by self-oxidation generate less reactive compounds. Even though a number of antioxidant enzymes are essential in the defense system, GSH-Px, catalase and SOD are considered among the important ones. These enzymes act directly on some free radicals, making them less reactive. However, they are not able to act on the highly reactive free radicals that are chiefly responsible for oxidative pathological processes such as hydroxyl and perhydryl radicals or peroxynitrite. GSH molecule of all the antioxidant molecules stands out as the most powerful protector of eukaryotic cells in the host defense against oxidative stress, acting upon several diverse mechanisms. Autonomously, the secretion of tumor necrosis factor-alpha (TNF-α) seems to induce oxidative stress through modulation of GSH metabolism, playing a key function in malaria physiopathogenesis. (Laurence et al, 2002).

**2.17 Mechanisms of Oxidative Stress in the Human Host**

Oxidative stress is caused by an imbalance between the pro-oxidant attacks and the antioxidant defense in animals. Particularly, RBCs in particular are susceptible to oxidative damage: 1) because as an oxygen carrier, RBCs are uninterruptedly exposed to high oxygen tension, 2) because RBCs have no capacity to repair their damaged components, and 3) because their membrane components are susceptible of lipid peroxidation . On the other hand, normal RBCs have a series of antioxidants, such as superoxide dismutase, catalase, glutathione peroxide, nicotinamide-adeninedinucleotide phosphate, nicotinamide-adeninucleotide, glutathione, glutathione reductase, capable of hydrolyzing the oxidatively modified proteins and preventing a degree of the adverse damages by the oxidative stress. The absence of these antioxidants often makes many microbial pathogens more vulnerable to oxygen radicals induced by the immune response (from monocytes and neutrophils) to the infection. In contrast, there are reports that the parasitic infection also induces the oxidative stress in the RBC environment. The parasites are considered to induce the production of reactive oxygen species by depleting these defense components of RBC as above. It would be important to investigate the roles increased oxidative stress serves in killing intraerythrocytic parasites or cause damage to host RBC or surrounding tissues. Free radicals and other reactive species are constantly generated in vivo and cause oxidative damage to biomolecules. Deoxyribonucleic acid (DNA) is probably the most significant target of oxidative attack. Oxidative damage to DNA by reactive oxygen species (ROS) may result in base modification, sugar damage, strand break, and DNA protein cross links. DNA Comet assay, (alkaline version in particular), is a popular method for the analysis of DNA damage. DNA damages consist of strand breakage, alkali labile sites and incomplete excision repair sites. The direct DNA-breaking can be estimated by alkaline elution, nick translation and also by the alkaline single cell gel-electrophoresis (SCGE) . Of these, modification of guanine by hydroxyl radicals at the C-8 site, frequently estimated as 8-OhdG is the most commonly studied lesion. Urinary excretion of 8-OhdG repair product from oxidative DNA modification by excision enzymes is an in vivo measure of overall oxidative DNA damage. where it also prevents the propagation of free radicals reactions as an effective antioxidant. Although ascorbic deficiency can contribute to anemia because lack of the antioxidants increases potential oxidant damage to erythrocyte, several animal studies have reported an apparent protective role of vitamin C deficiency in malaria. The absence of this antioxidant makes the parasite more vulnerable to oxygen radicals‘reactions from the immune response to Plasmodium infection.

 Biological significance of antioxidant like ascorbic acid (vitamin C) in human health. Vitamin C is a class of nutrient that are essentially require by the body for its various biochemical and physiological processes. Mostly, the human body does not synthesize them, therefore, they must be supplied by the diet in the required amount.. Vitamin C (ascorbic acids) is a water-soluble antioxidant; it was first isolated in 1928, by the Hungarian biochemist and unlike animals/humans cannot synthesize vitamin C rendering its ingestion from exogenous supplement / diet necessary. It has proposed that cause of human inability to synthesize ascorbic acids is the absence of the active enzyme,l-gulonolactone oxidase from the liver( burns, 1959).

A nutritional status is a condition of a person that is influenced by the intake and utilization of nutrient .A patient that is diagnosed with malaria / anaemia needs a nutritious diet to maintain a good health and when the body receives all the nutrient in appropriate amount so as to meet the needs of the body, then a normal nutritional status will achieve. it is necessary to eat foods that also include vitamins that will help your body retain iron

**2.18 Treatment of Malaria /Anemia and Management of Oxidative Stress**

**Antioxidant**

An antioxidant can be define as any substance that prevent damage to cellular components arising as a consequence of chemicals reactions involving free radicals. Antioxidants are molecules that inhibit or quench free radical reactions and delay or inhibit cellular damage (Adam-Vizi,, (2005). 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 (Adam-Vizi. (2005). The major enzymatic antioxidants are superoxide dismutase, catalyse and glutathione peroxidase.

Superoxide dismutase exists as a copper, zinc-enzyme (SOD1) that is found in the cytoplasm or a manganese containing enzyme that is located in mitochondria (SOD2). These enzymes catalyze the one-electron dismutation of superoxide (O2·) to hydrogen peroxide (2O2 + 2H+ → H2O2 + O2). Catalyse is an iron-dependent enzyme that directly decomposes hydrogen peroxide to water (2H2O2 → 2H2O + O2). Furthermore, glutathione peroxidases (GPXs) are a family of enzymes that reduce a variety of organic and inorganic hydroperoxides to the corresponding hydroxyl derivatives in the presence of glutathione (GSH). In this process, GSH is converted to an oxidized disulfide (2GSH + H2O2 → GS-SG + 2H2O).

The other way of categorizing the antioxidants is based on their solubility in the water or lipids. The antioxidants can be categorized as water-soluble antioxidants. The water-soluble antioxidants (e.g. vitamin C) are present in the cellular fluids such as cytosol, or cytoplasmic matrix.

**The role of superoxide dismutase**

**Superoxide dismutase** (**SOD**, [EC](https://en.wikipedia.org/wiki/Enzyme_Commission_number) [1.15.1.1](https://enzyme.expasy.org/EC/1.15.1.1)) is an [enzyme](https://en.wikipedia.org/wiki/Enzyme) that alternately catalyzes the [dismutation](https://en.wikipedia.org/wiki/Dismutation) (or partitioning) of the [superoxide](https://en.wikipedia.org/wiki/Superoxide) (O2−) [radical](https://en.wikipedia.org/wiki/Radical_%28chemistry%29) into either ordinary molecular [oxygen](https://en.wikipedia.org/wiki/Oxygen) (O2) or [hydrogen peroxide](https://en.wikipedia.org/wiki/Hydrogen_peroxide) (H2O2). Superoxide is produced as a by-product of oxygen metabolism and, if not regulated, causes many types of cell damage. Hydrogen peroxide is also damaging and is degraded by other enzymes such as [catalase](https://en.wikipedia.org/wiki/Catalase). Thus, SOD is an important [antioxidant](https://en.wikipedia.org/wiki/Antioxidant) defense in nearly all living cells exposed to oxygen. One exception is [*Lactobacillus plantarum*](https://en.wikipedia.org/wiki/Lactobacillus_plantarum) and related [lactobacilli](https://en.wikipedia.org/wiki/Lactobacillus), which use a different mechanism to prevent damage from reactive (O2−).

**The role of catalase**

Catalase is a common [enzyme](https://en.wikipedia.org/wiki/Enzyme) found in nearly all living organisms exposed to oxygen (such as [bacteria](https://en.wikipedia.org/wiki/Bacteria), plants, and animals). It catalyses the decomposition of [hydrogen peroxide](https://en.wikipedia.org/wiki/Hydrogen_peroxide) to [water](https://en.wikipedia.org/wiki/Water) and [oxygen](https://en.wikipedia.org/wiki/Oxygen). It is a very important enzyme in protecting the cell from [oxidative damage](https://en.wikipedia.org/wiki/Oxidative_stress) by [reactive oxygen species](https://en.wikipedia.org/wiki/Reactive_oxygen_species) (ROS). Likewise, catalase has one of the highest [turnover numbers](https://en.wikipedia.org/wiki/Turnover_number) of all enzymes; one catalase molecule can convert millions of hydrogen peroxide molecules to water and oxygen each second. Catalase is a [tetramer](https://en.wikipedia.org/wiki/Tetrameric_protein) of four polypeptide chains, each over 500 [amino acids](https://en.wikipedia.org/wiki/Amino_acid) long.[[7]](https://en.wikipedia.org/wiki/Catalase#cite_note-Boon_a-7) It contains four iron-containing [heme](https://en.wikipedia.org/wiki/Heme) groups that allow the enzyme to react with the hydrogen peroxide. The optimum [pH](https://en.wikipedia.org/wiki/PH) for human catalase is approximately 7, and has a fairly broad maximum: the rate of reaction does not change appreciably between pH 6.8 and 7.5. The pH optimum for other catalases varies between 4 and 11 depending on the species. The optimum temperature also varies by species.

**Vitamin C (Ascorbic acid)**

A high percentage of vitamin C is to be found in all greens as well as in many fruits and fresh vegetables, but animal tissue can only store this vitamin in minute quantities. Vitamin C helps form and maintains the "cementing" materials that hold body cells together and strengthen the walls of blood vessels, and it aids in healing wounds. Ascorbic acid participates in the synthesis of certain hormones and in cellular respiration. (Hill, 2006).



Fig 3 vitamin C

Source [www.wikipedia/file/vitamin c.jpg](http://www.wikipedia/file/vitamin%20c.jpg)

**2.18.1 Deficiencies**

Scurvy is the name for a vitamin deficiency and scurvy happens when there is a lack of vitamin C or ascorbic acid. It can lead to anaemia and vitamin deficiency anemia occurs when your body doesn’t have enough of the vitamins needed to produce adequate number of healthy red blood cells. Red blood cells carry oxygen from lungs throughout your body. If your diets is lacking in certain vitamin like ascorbic acids, it automatically will lead to vitamin deficiency anaemia and the deficiency will develop rapidly because the body can’t properly absorb that nutrient from food you eat. Vitamin C deficiency is also possible if something impairs your ability to absorb vitamin C from food. For instance, soaking impairs your body’s ability to absorb vitamin C; (Hill, 2006).

**2.18.2 Metabolism**

Ascorbic acid is metabolized in the liver, and to some extent in the kidney, in a series of reactions. The principal pathway of ascorbic acid metabolism involves the loss of two electrons. The intermediate free radical reversibly forms dehydroascorbic acid, leading to the irreversible formation of the physiologically inactive 2,3-diketogulonic acid Diketogulonic acid may be either cleaved to oxalic acid and threonic acid, or decarboxylated to carbon dioxide, xylose, and xylulose, leading eventually to xylonic acid and lyxonic acid. All of these metabolites and ascorbic acid itself fare excreted in the urine. (Hill, 2006).

**2.18.3 Food Sources**

Ascorbic acid is widely distributed in nature, mostly rich in fresh fruits and leafy vegetables such as guava, mango, papaya, cabbage, mustard leaves and spinach. (Hill, 2006).

Animal sources of this vitamin such as meat, fish, poultry, eggs and dairy products contain smaller amounts and are not significant sources. Most food–based dietary guidelines are similar in that all recommend consumption of 5 servings of fruits and vegetables daily. If this recommendation is followed, daily intake of ascorbic acid will be 210 to 280 mg, depending on food content factors (Hill, 2006).. Ascorbic acid is the least stable of all vitamins and is easily destroyed during processing and storage. Juices are good foods to be fortified with ascorbic acid because their acidity reduces ascorbic acid destruction. Exposure to oxygen, prolonged heating in the presence of oxygen, contact with minerals (iron and copper) and exposure to light are destructive to the ascorbic acid content of foods.

**2.18.4 Infants Requirement**

Human milk is recognised as the optimal milk source for infants at least throughout the first year of life. It is recommended as the sole nutritional milk source for infants during the first 4 to 6 months of life. IOM (2000) estimated the average intake (AI) for infants based on the average volume of milk intake of 780 ml and an average concentration of ascorbic acid of 50 mg/l in human milk. For infants 0-6 months, 40 mg per day was the estimated AI and for the 7-12 months infants, the AI was 50 mg per day, taking into consideration the amount of ascorbic acid from solid foods consumed at this stage.

The FAO/WHO expert consultation (FAO/WHO, 2002) estimated the mean ascorbic acid concentration in human mature milk as 40Mg/l. However, it was felt that the amount of ascorbic acid in human milk appears to reflect maternal dietary intake rather than the infant’s needs. Moreover, it was noted that 8 mg/day of ascorbic acid is sufficient to prevent scorbutic signs in infants. The Consultation therefore arbitrarily set the recommended intake for infants aged 0-6 months at 25 mg/day. The recommended intake for older infants was gradually increased to 30 mg per day.

RNI for infants 0 – 5 months 25 mg/day 6 – 11 months 30 mg/day

**2.18.5 Children and Adolescents**

No data were available on which to base an estimated average requirement (EAR) for children 1 through 18 years of age. Thus, the IOM (2000) estimated the EARs and RDAs for children on the basis of relative body weight.

Recommended Nutrient Intakes for Malaysia 2005

The FAO/WHO (2002) recommended intakes for ascorbic acid for children and adolescents were gradually increased from the recommended intake for infants. In deciding on recommended intake for older children, e.g adolescents, the TSC considered the possible role that ascorbic acid can play in reducing the high prevalence of iron deficiency anaemia in the country (Hill, 2006). had observed that the additional intake of at least 25 mg ascorbic acid promotes absorption of soluble non-haem iron. In addition, recent studies have pointed towards a possible antioxidant role for ascorbic acid, ie ability to scavenge reactive oxidants in activated leucocytes, lung, gastric mucosa and to protect against lipid peroxidation. The TSC therefore decided to increase the amount recommended by FAO/WHO (2010) by 25 mg ascorbic acid per day to all age groups from children 10 years and above.

RNI for children 1 – 3 years 30 mg/day 4 – 6 years 30 mg/day 7 – 9 years 35 mg/day

RNI for adolescents Boys 10 - 18 years 65 mg/day Girls 10 - 18 years 65 mg/day

**2.18.6 Adults**

The classic disease of severe ascorbic acid deficiency, scurvy, is now rare in most countries. Other human experimental data that can be utilised to set a ascorbic acid requirement, based on a biomarker other than scurvy, are limited. The IOM (2000) recommended intakes of ascorbic acid are based on an amount of the vitamin that is thought to provide antioxidant protection as derived from the correlation of such protection with neutrophil ascorbate concentrations. It is however recognised that there are no human data to directly quantify the dose-response relationship between ascorbic acid intake and in vivo antioxidant protection.

Based on ascorbic acid intakes sufficient to maintain near-maximal neutrophil concentrations with minimal urinary loss, IOM (2000) set an EAR of 75 mg/day of ascorbic acid for men. Based on this, and assuming a coefficient of variation of 10%, RDA for ascorbic acid for men was computed to be 120% of estimated requirement or 90 mg/day. Since no similar data were available for women, it is assumed that women will have lower requirement due to their smaller lean body mass, total body water, and body size. The RDA for women was thus set at 75 mg/day.

The IOM noted that at a ascorbic acid intake of 90 mg/day, the plasma ascorbate concentration reaches 50 µmol/l which has been shown to inhibit LDL oxidation in vitro systems. Although it is not known whether ascorbic acid prevents LDL oxidation in vivo, if it does this might be relevant in the prevention of heart disease. Also, since neutrophils 105Ascorbic Acid (Vitamin C) are at 80 percent saturation at an EAR of 75 mg/day, this should potentially protect intracellular proteins from oxidative injury when these cells are activated during infectious and inflammatory processes.

FAO/WHO (2002) calculated the dietary intake from physiologic requirements. At saturation the whole body content of ascorbate in adult males is approximately 20 mg/kg, or 1500 mg. Clinical signs of scurvy appear when the whole body content falls below 300–400 mg, and the last signs disappear when the body content reaches about 1000 mg. In these experiments, ascorbate in the whole body was catabolised at an approximate rate of 2.9 percent/day.

There is a sigmoidal relationship between intake and plasma concentrations of ascorbic acid. At low doses, dietary ascorbic acid is almost completely absorbed, but over the range of usual dietary intakes (30–180 mg/day), absorption may decrease to 75 percent because of competing factors in the food.

A body content of 900 mg falls halfway between tissue saturation and the point at which clinical signs of scurvy appear. Assuming an absorption efficiency of 85 percent, and a catabolic rate of 2.9, the average intake of ascorbic acid can be calculated as: 900 x 2.9/100 x 100/85 = 30.7 mg/day, which can be rounded off to 30 mg/day.

The recommended nutrient intake (RNI) would therefore be:

900 x (2.9 + 1.2)/100 x 100/85 = 43.4 mg/day, which can be rounded off to 45 mg/day.

No turnover studies have been done in women, but from the smaller body size and whole body content of women, requirements might be expected to be lower. However, in depletion studies plasma concentrations fell more rapidly in women than in men. FAO/WHO (2002) therefore made the same recommendation for non-pregnant, nonlactating women as for men.

An intake of 45 mg/day will ensure that measurable amounts of ascorbate will be present in the plasma of most people and will be available to supply tissue requirements for metabolism or repair at sites of depletion or damage. A whole body content of around 900 mg of ascorbic acid would provide at least 1 month’s safety interval, even for a zero intake, before the body content falls to 300 mg.

It has been reported that elderly people generally have lower plasma and tissue ascorbate levels than young people, often because of poor dentition or mobility problems. However, FAO/WHO (2002) felt that the requirements of elderly people do not differ substantially from those of younger people in the absence of pathology, which may influence absorption or renal functioning. The recommended intakes for the elderly are therefore the same as those for adults (45 mg/day).

For reasons already mentioned above for the adolescents, the TSC on Vitamins has proposed that 25 mg per day ascorbic acid be added on to the FAO/WHO (2002) recommended intake of 45 mg per day for all groups above 10 years of age.

RNI for adults Men 19 – 65 years 70 mg/day Women 19 – 65 years 70 mg/day

RNI for elderly Men > 65 years 70 mg/day Women > 65 years 70 mg/day.

**2.18.7 Pregnancy and Lactation**

During pregnancy there is a moderate extra drain on ascorbic acid, particularly during the last trimester. It has been reported that 8 mg/day of ascorbic acid is sufficient to prevent scorbutic signs in infants aged 4–17 months. FAO/WHO (2002) therefore provided an extra 10 mg/day throughout pregnancy, to bring the recommended intake to 55 mg/day. This enables reserves to accumulate to meet the extra needs of the growing foetus in the last trimester.

During lactation, it has been estimated that 20 mg/day of ascorbic acid is secreted in milk. For an assumed absorption efficiency of 85 percent, an extra 25 mg will be needed by the mother. FAO/WHO (2002) therefore recommended that the RNI should be set at 70 mg to fulfil the needs of both the mother and infant during lactation. For the same reasons mentioned for the adolescents, the TSC for Vitamins suggested to add an additional 25 mg per day of ascorbic acid to the FAO/WHO (2002) recommended intake for pregnant and lactating women.

RNI for Pregnancy 80 mg/day Lactation 95 mg/day

Discussions on revised RNI for Malaysia

The RNI values for ascorbic acid for Malaysia, adapted from FAO/WHO (2002), but with the addition of 25 mg per day for all age groups above 10 years of age, are also the same as those adopted by the Working Group for the Harmonisation of RDAs in SE Asia (2002). The SEA Group also decided to provide for an additional amount mentioned. Appendix 10.1 provides a summary of these revised RNI, compared with the previous Malaysian RDI the FAO/WHO (2002) recommendations and the values recommended by IOM (2000).

**2.19 The Roles of Ascorbic Acid in Biological Pathways**

Free radicals are produced through biological processes and in response to exogenous stimuli, and controlled by various enzymes and antioxidants in the body. Oxidative stress occurs when free radical formation exceeds the ability to protect against them, what can lead to tissue injury after trauma, inflammatory events and chronic conditions, such as a degenerative disease like malaria/anaemia can be elucidate by vitamin C. often referred to as “antioxidant vitamins like vitamin C percisely”, have been suggested to limit oxidative damage in humans, thereby lowering the risk of certain chronic diseases.

ascorbate free radical may be the primary product of the oxidation. The catecholamine biosynthesis occurs in the adrenal glands and the brain, both with relatively large amounts of ascorbic acid. Ascorbic acid protects catecholamines by direct chemical interactions and by elimination of adrenocrome, a toxic product of catecholamine oxidation, which has been linked to certain mental diseases.

The hydroxylation of tyrosine to catecholamines and the hydroxylation of phenylalanine to tyrosine seem to involve the folic acid derivative tetrahydrobiopterin as an electron carrier, and the recycling of ascorbic acid.

Ascorbic acid may function to restore this substrate from the oxidized dihydrobiopterin. It has been suggested that dopamine-β-hydroxylase works in conjunction with monodehydroascorbate reductase to recycle tetrahydrobiopterin.

The synthesis of serotonin, a neurotransmitter and vasoconstrictor, involves the hydroxylation and decarboxylation of tryptophan. The initial hydroxylation step, catalyzed by tryptophan hydroxylase, is thought to require ascorbic acid. The cosubstrate for the hydroxylase is tetrahydrobiopterin. Again, it has been suggested that ascorbic acid is able to restore this substrate from its oxidized form, dihydrobiopterin.

**CHAPTER THREE**

**3.0 Materials and Methods**

**3.1 Consumables**

* EDTA tubes
* Methylated spirit
* Cotton wool
* Pipette tips
* Hand gloves
* Distilled water
* 5-10ml syringe

**3.2 Equipment**

* Conical flasks
* Pipette pumps
* Weighing balance
* Centrifuge
* UV spectrophotometer (UH4150 Hitachi Japan)
* Beakers
* Reagent bottles
* Spatula
* Stirring rod
* Incubator
* HB Haemoglobin test kit (Plastic industries ltd India).
* Microscopic slides
* Standard diagnostics (SD) Rapid Test kit (ELISAS inc. Australia)

**3.3 Reagents**

* 10% trichloroacete acid(TCA)
* 0.2M Dinitrophenylhydrazine (DNPH)
* 1ML Sulphuric acid
* 0.2ml Ascorbic acid
* 0.4ml hydrogen peroxide
* 2ml dichromate-acetic acid
* 8mM paarogallol
* 0.02M phosphate buffer

**3.4 Study Population and Design**

This study was a prospective cross-sectional carried out at the Enugu State University Teaching Hospital (ESUTH) Enugu involving malaria patients visiting the hospital. A total number of 101 patients infected with malaria parasite were recruited for the study.

**3.5 Study Duration**

This one month study was carried out between May to early June 2018 at Enugu state University of Science and Technology teaching hospital Parklane, Enugu state.

**3.6 Inclusion / Exclusion Criteria**

Outpatients were screened for malaria infection and only patients with uncomplicated malaria infection will be recruited for the study. Patients with severe malaria or critical health complication who are admitted at the hospital will be excluded from the study. Also, pregnant and/ or women, breast feeding as well as patients with oxidative stress related diseases excluded from the study.

**3.7 Data Collection**

Questionnaire was used for the collection of baseline information of patients including age, sex, genotype, blood group, the duration of the disease, ethnic group, location, treatment administered and progress of treatment.

**3.8 Blood collection**

A total volume of 5ml of blood was collected from each patient of which 2.5ml will be transferred into anticoagulant tube ( EDTA) while the remain 2.5ml will be transferred into anticoagulant free tubes ( EDTA free tubes) for various analysis.

**3.9 Sample Collection**

After enrolling the participants, ten milliliters of blood sample of blood specimen were collected from subject (both test and control); thick and thin film were made, and both thin/ thick stained with 10%Giemsa and examined for malaria parasites. 5ml of the blood was dispensed into EDTA tubes and were properly swirled to avoid clotting. Also a volume of 5ml of blood was used to test antioxidant profile, catalyse activity, and superoxide dismutase. This sample will be collected once and interaction you will include reading of consent form, collection of data and sample will last for minutes. After study, will be discarded and will not be reused for any other purposed.

**3.10 Malaria Diagnosis by Rapid Diagnostic Test (RDT)**

Open the RDT package and remove the contents. The blood-transfer device it could be a capillary tube, straw, loop, pipette or other device is used to collect blood and transfer it to the test cassette.(Once the packet is opened, the ‘desiccant’ sachet which absorbs moisture from the atmosphere in the package should be discarded.) The test cassette is used to conduct the test. The square hole labelled ‘A’ is where you add the blood. The round hole labelled ‘B’ is where you add the buffer.

**3.10.1 Drawing blood with a Tube.**

Use the device EDTA tubes, pipette or other) to add the drop of blood to the sample window (square hole labelled with the letter A, The blood needs to reach and be absorbed by the pad at the base of the square hole. If the blood is mostly deposited on the plastic edges of the well, but does not reach the pad, the test will not work correctly. Deposit the blood in the correct place using the capillary tube, straw, loop, pipette or other. Adding too much or too little blood can cause the test to give an invalid result or be difficult to read.

Adding blood to the RDT cassette.

Add the buffer solution to the round hole labelled B. Hold the bottle vertically when adding the buffer solution. This ensures the correct drop size.



Adding the buffer solution , Wait for the correct duration of time (15 or 20 minutes) after adding buffer before reading the test results. Discard the blood-collection device (e.g. capillary tube) safely after use.

 Remove and discard your gloves at this time. To avoid possible contamination, the used gloves should be discarded in the non-sharps container before you do anything else.

### 3.10.2 How to Read and Interpret an RDT Test Result

The different possible results and what they mean are illustrated. 

### 3.11 Microscopic Test for Malaria

Malaria parasites being viewed under a microscope.

Microscopic diagnosis of malaria is done by a trained laboratory technician at health centre or hospital level. It is not your responsibility to do a microscope test, but this section will briefly explain it so you understand the technique.

Microscopic diagnosis involves taking a small amount of blood from the patient, staining it and looking at it under a microscope to check for malaria parasites. In most cases of malaria, microscopic examination of thick and thin films of finger-prick blood will reveal malaria parasites. Thick films are 20–40 times more sensitive than thin films for detecting Plasmodium parasites, and are particularly useful if the number of parasites is low. Thin smears are also useful as they can allow identification of particular Plasmodium species. The diagnostic accuracy relies on the quality of the blood smear and the experience of laboratory personnel.

Thick and thin blood smears was be prepared and stained with giemsa after which asexual malaria parasites will be counted against 200 leucocytes under the microscopy and the parasite density expressed as per 𝜇L of blood.

* 1. **Diagnosis of Haemoglobin Cone and Haematocrit**

Anaemia indices including haemoglobin (Hb) concentration, haematocrit, mean cell volume (MCV), mean cell haemoglobin (MCH), pack cell volume (PCV) and blood cell counts ( WBC & RBC counts, and WBC differential count will be done using auto analyser.

**Procedure for HB Test**

* Insert the code chip into the meter and code the meter correctly.
* Remove the strip from the closed canister and use it as soon as possible. Immediately close the canister tightly after removing the required number of strip(s).
* Wait for the meter to flash the strip symbol. Insert the strip completely into the strip channel in the same direction as the arrows printed on the test strip until the white edge above the black line on the test strip is no longer visible.
* Wipe away the first drop of blood, collect 10𝜇L of the second drop of capillary blood using a capillary transfer tube or pipette. Hold the tube slightly downward and touch the tip of the capillary transfer tube to the blood drop. Capillary action will automatically draw the sample to the fill line and stop.
* While the meter is flashing the blood drop symbol, align the tip of the capillary transfer tube with the specimen application area of the strip to apply the blood (10uL). 3 dashed lines will appear on the meter to show the test is in progress.
* Read the result on the screen after 15 seconds. Refer to testing in the users for detailed test procedures.
* **Note**: make sure the blood covers the air vent of the tube or it will be hard to squeeze blood out. Do not squeeze the capillary transfer tube while collecting specimen.

**3.13 Determination of Vitamin C**

The level of vitamin C in the plasma was determined using the method of Vitamin C presents in the plasma forms a colored product on treatment with 2, 4 dinitrophenylhydrazine in the presence of copper sulfate. The absorbance of the colored product was measured at 520 Nm measurement of ascorbic in blood:

**Procedure**

Test tubes were labelled test, standard and blank. In the test tube labelled test, 200uL of blood was added, 200uL of concentrated ascorbic acid was added in test tube labelled control standard and 200uL distilled water was added in test tube labelled blank. 0.2ml of trichloroacete acid was added in all test tubes, 0.2ml of dinitrophenylhydrazine. They were mixed well and incubate at 37’c for 3hours, after incubating, 1000uL of sulphuric acid was added and mixed again and kept in a room temperature at 30 minutes. Absorbance was read for 520 nm against the blank using a spectrophotometer.

**3.14 Determination of Catalase Activity**

The method described by Pari and Latha (2004) was adopted for the determination of catalase activity. Liver tissue was obtained and homogenized in 0.01 mol/L phosphate buffer (pH 7.0) and the homogenate was centrifuged at 5 000 r/min. A volume of 0.1 mL of liver homogenate (10% w/v) was added to a reaction mixture containing 0.4 mL of hydrogen peroxide (0.2 mol/L) and 1 mL of 0.01 mol/L phosphate buffer (pH 7.0). Finally, 2 mL of dichromate-acetic acid reagent (5% potassium dichromate prepared in glacial acetic acid) was added to the mixture to stop the reaction. The absorbance was taken at 620 nm and the percentage inhibition was calculated using the equation:

Catalase inhibition (%) = [(Normal activity – Inhibited activity)/

(Normal activity)] × 100%

Where, Normal activity was hydrogen peroxide + phosphate buffer; Inhibited activity was hydrogen peroxide + phosphate buffer + liver homogenate.

**3.15 Determination of Superoxide Dismutase (SOD) Activity:**

SOD activity will be quantified according to Marklund and Marklund (1974) method with slight modifications. Homogenized blood sample (50 µl) will be transferred into a tube containing 450 µl of 4 mM pyrogallol and 500 µl of 20 mM phosphate buffer (pH 6.6). The absorbance was taken at 450 nm for 3 minutes against a blank preparation containing distilled water instead of sample in pyrogallol and phosphate buffer. SOD activity was expressed as U/mg. One unit of SOD activity is defined as that amount of SOD required to cause 50% inhibition of pyrogallol autooxidation per 3ml of assay mixture.

**3.16 Statistical Analysis**

Data was analyzed with Statistical Package for Social Science (SPSS) version 16. Genotype and allele frequencies will be compared using the χ2 statistics or the fisher’s exact test. Continuous variables will be compared using parametric tests (independent sample t-test and ANOVA with post hoc multiple comparison by Dunn-Sidak test). An odd ratio was calculated by logistic regression adjusting for age. A p-value less than 0.05 were considered statistically significant.

**CHAPTER FOUR**

**4.0 Results**

Among anaemia patients, 17 (16.8%) were male and 41 (40.6%) were female. Among the non- anaemic patients, 13 (12.9%) were male and 30 (29.7%) were female. There was no significant relationship between anaemia and gender of patients as the (p = 0.920). The result is summarised in the Table 3 below.

**Table 3: Gender distribution of study population**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Characteristics(n =101) |  | **Anaemic****Patients** | **Non-Anaemic patients** | *Total* | *χ2* | *p-value* |
| Study participants | Male | 17 (16.8%) | 13 (12.9%) | 30 (29.7%) | 0.010 |  |
| Sex | Female | 41 (40.6%) | 30 (29.7%) | 71 (70.3%) |  | 0.920 |
|  | Total |  | 43 (42.6%) | 101 (100.0%) |  |  |

The above shows that baseline characteristic of the study are suitable as the age indicates a slight difference, but there was no significant difference that show in the height, weight, systolic blood pressure, diastolic blood pressure, pulse level of anaemic and non anaemic patients making their values suitable for the study; And it is summarized in the Table 4 below.

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

|  |  |  |  |
| --- | --- | --- | --- |
| Characteristics (n =101) | **Anaemic** **Patients** | **Non-anaemic****Patients** | *p-value* |
| Age (years) | 31.34±2.02 | 40.05±2.92 | 0.013 |
| Height (m) | 9.86±4.11 | 5.49±0.24  | 0.414 |
| Weight (kg) | 34.58±4.82 | 27.53±0.49 | 0.324 |
| SBP (mmHg) | 120.36±2.42 | 124.43±2.87 | 0.282 |
| DSP (mmHg) | 78.18±1.45 | 79.95±1.811 | 0.441 |
| Pulse (mg/dL) | 106.79±17.19 | 82.74±1.99 | 0.235 |

The comparison of anaemia and oxidative stress indices among patient’s shows that the haemoglobin and haemtocrit concentration were significantly lower in anaemic patients compare to non anaemia patients with the p-value (<0.001). The vitamin C and SOD as there was no significant difference between the anaemia and non anaemia patients. The catalase showed to be influenced by anaemia as the catalase is higher in anaemia patients than non anaemia patients and it is summarized in Table 5 below.

**Table 5: Comparison of anaemia and oxidative stress indices among patients**

|  |  |  |  |
| --- | --- | --- | --- |
| Characteristics (n =101) | **Anaemic patients** | **Non-anaemic****Patients** | *p-value* |
| Haemoglobin (mg/dl) | 10.33±0.194 | 13.66±0.24 | <0.001 |
| Haematocrit (%) | 30.38±0.57  | 40.07±0.73 | <0.001 |
| Vitamin C (mg/dL) | 57.83±1.75 | 60.81±1.44 | 0.212 |
| Catalase (U) | 1.45±0.03 | 1.35±0.03 | 0.039 |
| SOD (10-1U) | 3.60±0.38 | 3.13±0.45 | 0.423 |

The relationship between anaemia and malaria infection among patients showed that there was no significant relationship between anaemia and malaria as the anaemic patients were 58 (57.4%) higher compared to non anaemic patients 43 (42.6%) and it is summarized in Table 6 below.

Table 6: **Relationship between anaemia and malaria infection among patients**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Characteristics |  | AnaemicPatients | Non-Anaemic patients | Total | *χ2* | *p-value* |
| Malaria | **Present** | 49 (48.5%) | 37 (36.6%) | 86 (85.1%) |  |  |
| **Absent** | 9 (8.9%) | 6 (5.9%) | 15 (14.9%) | 0.048 | 0.827 |
| **Total** | 58 (57.4%) | 43 (42.6%) | 101 (100.0%) |  |  |

There was no significant interaction between anaemia and malaria influence base the study parameters which are the Haemoglobin, Haemocrit, Vitamin C, SOD, Catalase Concentrations and it is summarized in Table 7 below.

**Table 7: Interaction between anemia and malaria on study parameters**

|  |  |  |  |
| --- | --- | --- | --- |
| Characteristics (n =101) | AnaemicPatients | Non-anaemicPatients | 2 Way ANOVA *(p-value)* |
| **Malaria****Patients** | **Non-Malaria****Patients** | **Malaria****Patients** | **Non-malaria****patients** | **Anaemia status** | **Malaria status** | **Anaemia x Malaria** |
| Haemoglobin conc. (mg/dL) | 10.30±0.22 | 10.55±0.55 | 13.77±0.25 | 13.0±0.63 | 0.000 | 0.561 | 0.261 |
| Haematocrit (mg/dL) | 30.31±0.66 | 31.0±1.62 | 40.35±0.75 | 38.33±1.87 | 0.000 | 0.621 | 0.313 |
| Vitamin C | 58.29±1.69 | 55.05±4.19 | 60.73±1.95 | 61.31±4.84 | 0.210 | 0.701 | 0.583 |
| Catalase | 1.43±0.03 | 1.52±0.08 | 1.36±0.04 | 1.29±0.09 | 0.025 | 0.945 | 0.230 |
| SOD (10-1U) | 3.73±0.42 | 2.79±.1.07 | 3.25±0.48 | 2.27± 1.26 | 0.573 | 0.279 | 0.976 |

**CHAPTER FIVE**

* 1. **Discussion**

Anaemia is a blood disorder problem in the world, which approximately, 1 billion people worldwide are affected by anaemia deficiency. Findings from this study showed that 58(57.4%) were anaemia while 43(42.6%) were non anaemic. The haemoglobin & haematocrit concentration level were significantly lower in anaemia patients, compare to non anaemia patient, this may be due to the fact that anaemia can result as a lower level of level; previous study have confirmed that anaemia is due to haemoglobin level concentration (Becker *et al*., 2004).

Anaemia can be cause by malaria and oxidative stress. In this study, there was no relationship between aneamia and malaria. As the results showed there were no significant difference base on (p = 0.827).

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. (Becker *et a*l, 2004). It was observed that erythrocytes infected with *Plasmodium. falciparum* produced OH• radicals and H2O2 about twice as much compared to normal erythrocytes. Higher level of these free radicals can lead to oxidative stress. (Becker *et al,* 2004). 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 way**.** Antioxidants are molecules that inhibit or quench free radical reactions and delay or inhibit cellular damage (Adam-Vizi.2005). When this free radicals are higher, the antioxidant measure up to prevent them. It was observed in this study that cataslase was significantly higher in anaemic patients compared to non-anaemic patients. However, Vitamin C and SOD showed no significant difference between the anaemia and non anaemia patients.Previous study have also showed that setting antioxidant such as catalase many come up to remove free radicals when people are anaemia and prevent further anaemia in a living system by an act of antioxidant are the host defenses system that prevent damage to cellular components arising as a consequence of chemicals reactions involving free radicals.

* 1. **Conclusion**

Findings from this study showed that there was no relationship between anaemia and malaria to influence antioxidant status of patient visiting ESUTH, Enugu as Though there was an association between malaria and anaemia.

This may be due to the elucidation of results which showed from the analysis that there was no significant difference between the studied parameters. In order words this parameters does not necessary interacts together to influence malaria and anaemia.

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