EFFECTS OF *MORINGA OLEIFERA* LEAF POWDER ON NUTRITIONAL STATUS AND MALARIA PARASITE REINFECTION IN CHILDREN UNDER 5 YEARS

BY

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CERTIFICATION PAGE

I hereby certify that this work "Effects of *Moringa oleifera* leaf powder on the nutritional status and malaria parasite reinfection in children under 5 years in Oba community, Nigeria" was carried out by Ogolo, Bernice A; NAU/PG/PhD/2009537002P, under the supervision of Prof Obioma C. Nwaorgu and has not been presented elsewhere for the award of any degree or certificate. All sources have been duly distinguished and appropriately acknowledged.

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APPROVAL PAGE

The dissertation titled "Effects of *Moringa oleifera* leaf powder on the nutritional status and malaria parasite reinfection in children under 5 years in Oba community, Nigeria" has been examined and approved as meeting the requirements for the award of Doctor of Philosophy (Ph.D.) degree in Public Health Parasitology in the Department of Parasitology and Entomology, Faculty of Biosciences, Nnamdi Azikiwe University Awka.

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DEDICATION

This work is dedicated to God for His guidance and protection throughout the period of this work

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ABSTRACT

Children below five years of age infected with malaria parasite often present symptoms of fever, loss of appetite and malnutrition and may die due to their inability to build up competent immune system. A longitudinal study to investigate the effects of *Moringa oleifera* leaf powder on the nutritional status and malaria reinfection in children 4-5 years in Oba community was carried out from May 2015 to May 2016. Ethical approval for the study was obtained from the Ethical committee of Nnamdi Azikiwe University Teaching Hospital Nnewi. Five hundred children (217 males and 283 females); 4-5 years old, were randomly selected by balloting from 10 out of 30 nursery schools in the community. The children were subdivided into two experimental groups A and B. Group A children were fed with jollof rice and National Agency for Foods Drug Administration and Control (NAFDAC) approved *Moringa oleifera* leaf powder, while Group B children were fed with jollof rice only, as a daily lunch pack for six months. The nutritional status of the children, malaria prevalence and haematological profiles, packed cell volume (PCV), haemoglobin (Hb), white blood cell count (Wbc), platelets, protein and iron were obtained at baseline and monitored at bimonthly intervals following feeding for six months. Nutritional status were assessed using anthropometry, malaria prevalence data were obtained using microscopy while haematological profiles were obtained using standard laboratory procedures. Children positive to malaria infection at baseline, 71(14.2%) were treated with Artemether/lumefantrine (Coartem) and reintegrated into the groups. None of the children fed with jollof rice and *Moringa oleifera* leaf powder was reinfected with malaria parasites within the study period. A malaria reinfection prevalence of 46 (18.4%) with a parasite intensity of 3% were recorded among the children fed with jollof rice only after two months following feeding, none was recorded in the fourth month, but at the sixth month a significant prevalence of 36 (14.4%) and a parasite intensity of 2 % were recorded among the group ($P<0.05$; χ^2 38.793). *Plasmodium falciparum* was the only malaria parasite species observed. Anthropometric indices increased significantly on the children fed on jollof rice with *Moringa oleifera* leaf powder with mean values of 21.14kg ± 2.62, 116.94cm ± 5.80 and 16.59cm ± 1.43 for weight, height and mid upper arm circumference (MUAC) respectively ($p<0.05$). There was also a significant increase in the haematological parameters with mean values of 32%, ± 0.02, 10.78g/dl ± 0.70, 6.17cell/mcl ± 0.87, 286.83/mcl ±78.20, 73.45g/l ± 8.00 and 77.3µ/dl ± 16.3 for packed cell volume, haemoglobin, white blood cell count, platelets, protein and iron respectively among the children fed with jollof rice and *Moringa oleifera* leaf powder as opposed to those fed with jollof rice only. *Moringa oleifera* leaf powder significantly reduced the frequency of malaria reinfection, improved nutritional status and haematological profiles of the children. It is recommended that *Moringa oleifera* leaf powder be included in the school feeding programme as part of nutritional intervention for improvement of nutritional status of children less than five years old for reducing the global burden of malaria disease in the age group.

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CHAPTER ONE

INTRODUCTION

1.1 Background

Malaria is a preventable and an easily treatable disease, yet approximately 800,000 children die from it in Africa every year (Rowe *et al,* 2006). It is the commonest reason for hospitalization among children under five years and a leading contributor to the widespread problem of anaemia (Carneiro *et al,* 2006). Malaria continues to be a major public health problem in 97 countries and territories in the tropics and subtropics. Globally, approximately 214 million cases of malaria occur annually and 3.2 billion people are at risk of infection (WHO, 2015). Approximately 438,000 deaths were attributed to malaria in 2015, particularly in sub-Saharan Africa, where an estimated 90 % of all malaria deaths occur (WHO, 2015). Malaria was ranked in 2004 as the eighth highest contributor to the global disease burden and the second highest contributor in Africa (WHO, 2002). It was responsible for 2.9% of the global disability-adjusted life years (DALYs) lost and 10.1% of the total DALYs lost in Africa (Breman *et al,* 2004). As a critical target of the Millennium Development Goals, in 2005, the World Health Assembly established a goal of reducing malaria cases and deaths by 75 % between 2005 and 2015 (WHO, 2005). Hence, over the past decade, there has been greatly renewed interest in research and innovations in diagnostic methods, drugs and vaccines, and the development of control measures to eradicate malaria (Korenromp *et al*, 2013). As a result, between 2000 and 2013, the incidence rates of malaria fell by 30 % globally and by 34 % in Africa (Murray *et al*, 2014).

In Nigeria, malaria consistently ranks among the five most common causes of death in children (Nwaorgu and Orajaka, 2011). Female anopheles mosquitoes are vectors of malaria parasite and the dominant species in Nigeria are *Anopheles gambiae* and *Anopheles funestus* group (Aribodor, 2012). Malaria is endemic in Nigeria and the estimated mortality rate for children under five years between 1988 to 1992 was 729 per 100, 000 making it a leading cause of morbidity and mortality (WHO, 2010). It is estimated that over 50% of Nigerians suffer at least one bout of malaria every year (Semba and Bloem , 2001) and as much as 60% of school children's learning may be impaired by malaria (Booth and Maclean 2001). Federal Ministry of Health, (2001) reported that malaria prevalence (notified cases) in 2000 was about 2.4 million and that malaria leads to 25% of infant mortality and about 132 billion naira is lost annually in the form of treatment cost, prevention and loss of work. Malaria cases in Nigeria fell gradually from 497.8

cases per 100,000 people in 2000 to 380.8 cases per 100,000 people in 2015 (World data Atlas, 2015). According to Nwaorgu and Orajaka (2011), *Plasmodium falciparum* is the predominant species found in the blood of children with malaria from 1 – 10 years old. The results of the comprehensive study of the malaria situation in Nigeria conducted across the six geographical zones have confirmed that malaria is a major cause of morbidity and mortality especially among vulnerable groups including pregnant women and children less than 5 years old (FMOH, 2001). Malaria imposes great burden on the country in terms of pains and trauma suffered by its victims as well as loss in outputs and cost of treatments (Onwujekwe *et al*, 2000).

Infection with both *Plasmodium falciparum* and *P. vivax* has been associated with impaired physical growth in children (Takakura *et al,* 2001). The direction of this association appears to be two-way, with malaria leading to compromised nutritional status which increases susceptibility to malaria infection. Catch-up growth has been observed following interventions focusing on malaria prevention in children. This is because malaria fever reduces appetite, and exacerbates malnutrition (Shiff *et al,* 1996), thus suggesting that malaria infection may play a role in the etiology of malnutrition, and the downward cycle of impaired development of mental functions (Galler *et al*, 2001).

Malaria and malnutrition are major causes of morbidity and mortality in children as malaria has the capacity to turn borderline nutritional deficiency into severe malnutrition (Muller and Krawinke, 2005). Malnutrition commonly affects all groups in a community, but infants and young children are the most vulnerable because of their high nutritional requirements for growth and development. If malaria is a major contribution of malnutrition, it may therefore be responsible for considerable indirect mortality that could potentially be reversed by effective malaria control (Nyakeriga *et al,* 2004). Populations in malaria endemic areas often live under conditions that predispose them to both malaria and malnutrition and because children have the highest risk for severe malaria, often they also have the highest risk for poor nutrition (Caulfield *et al,* 2004).

Malnutrition results from a poor diet or a lack of food when the intake of nutrients or energy is too high, too low, or poorly balanced (Nordqvist, 2017). According to UNICEF and WHO (2007), malnutrition refers to deficiencies, excesses or imbalances in a person's intake of energy and/or nutrients. The term malnutrition covers 2 broad groups of conditions. One is 'undernutrition' which includes stunting (low height for age), wasting (low weight for height),

underweight (low weight for age) and micronutrient deficiencies or insufficiencies (a lack of important vitamins and minerals). WHO/NCHS reference and prevalence of wasting and stunting have been widely used to characterize the nutritional status of populations (WHO, 1995). Reasons being that, wasting reflects a deficit in weight relative to height due to a deficit in tissue and fat mass, whereas stunting reflects a deficit in height relative to age due to linear growth retardation. Among the three methods of assessing nutritional status (i.e. clinical signs of malnutrition, biochemical indicators and anthropometry); anthropometry has an important advantage over other nutritional indicators. This is however, the recommended method of choice for nutritional surveys (De Onis, 2000).

Nutrition plays a major role in maintaining health while malnutrition appears to generate vulnerability to a wide variety of diseases and general ill health (Semba and Bloem, 2001). An undernourished child is not capable to build up appropriate immune response to malaria parasite infections due to reduction in T lymphocytes, impairment of antibody formation, decreased complement formation, atrophy of thymus and other lymphoid tissues (Scrimshaw and SanGiovanni, 1997). Malaria causes anaemia through cytokine-mediated suppression of haematopoeisis, and in addition, infection with *P. falciparum*, causes erythrocyte changes and vulnerability to clearance of erythrocytes (Biggs and Brown, 2001).

Malaria causes alterations in some biochemical and haematological parameters in both adults and children. This includes hypoglycaemia, lactic acidosis, dislipidaemia and raised serum transaminases (Ogbodo *et al*, 2008; Adeosun *et al,* 2007). Poor nutrition and micronutrient deficiencies play important roles in the pathogenesis of malaria and malarial anaemia; hence in rural areas with poor socio-economic burden and inevitable nutritional deficiencies, malarial parasitization of vulnerable children is on the increase (Crawly, 2004). According to Fishman *et al* (2004), large numbers of children less than five years old suffer and die of malaria due to nutritional inadequacies. However malaria illness episodes and deaths are entirely preventable with appropriate nutritional interventions.

1.2 Justification

Investigations of the relationship between nutritional status and malaria have concentrated on nutrients such as vitamin A, zinc and protein that have significant impact on them. Vitamin A

plays an essential role in the proper functioning of the immune system and is believed to be necessary for host resistance to malaria (Shankar, 2000). Zinc is a required element in basic biologic processes such as gene expression, cellular growth and differentiation (Macdonald, 2000). Deficiencies in these nutrients may also directly impact on the growth and development of children. This has been evidenced by the benefits of supplementation of children's diets with vitamin A, zinc, and protein-rich foods thus leading to improved mental function performance and physical growth (Villamor *et al,* 2002).

Moringa oleifera leaves contain very strong concentrations of vitamins A, C, B complex, iron, calcium, protein, zinc, selenium and all essential amino acids for the nutritional requirements of children and protection from ill health (Fuglie, 2001, Vanisha *et al,* 2003; Igwilo *et al,* 2011). *Moringa oleifera* tree is encountered in all ecological zones in Nigeria where it is in season all year round (Ozumba, 2008). The vitamin A found in *Moringa oleifera* leaf is four times the amount found in carrots (Fuglie, 2001) and has the potential of building immune systems and sustaining health. Young children that consume dried *Moringa oleifera* leaf powder on a regular basis increase their weight and overall health and adding one large spoonful (8g) of dried *Moringa* leaf powder to complementary foods three times each day will ensure that a child is getting a nutrition rich diet (USAID, 2012).
A daily dose of one rounded tablespoon (8 g) of *Moringa oleifera* leaf powder will satisfy about 14% of the protein, 40% of the calcium, 23% of the iron, 13% of the potassium, 7% of the Vitamin C and 100% of the vitamin A for a child under five years (Fuglie, 2001). A major advantage of *Moringa oleifera* leaf is the fact that it is a local resource found everywhere in various communities in Nigeria, this contrasts with other programmes designed to fight malnutrition which depends on imported products (like vitamin A supplement) and outside donor support which may not continue when there is donor fatigue (Ikpeze *et al*, 2010).

The increased child morbidity and mortality rates due to malaria and malnutrition in developing countries including Nigeria have been a cause of concern to public health practitioners in Nigeria. Areas endemic for malaria often have a high prevalence of micronutrient malnutrition and both share certain consequences, including iron and protein deficiencies, cognitive impairment and decreased school performance (Branca and Ferrari, 2002). Previous interventions by supplementation of childrens diet with Vit A, Zinc and protein rich foods led to

improved mental function performance and physical growth (Villamor *et al,* 2002). Due to resistance to some of the conventional drugs used for the treatment of malaria and the impact of malaria on health, it is therefore necessary to search for new, cheap and easily available nutrient that will boost nutritional status among children and reduce malaria attack (Dondorp, 2007). It is anticipated that using *Moringa oleifera* leaf powder rich in proteins, vitamins and minerals could improve the nutritional status of young children and reduce malaria morbidity and mortality. This is in view of the fact that well-nourished children have a better immune response and are more capable of withstanding and clearing malaria infection.

1.3 Statement of the Problem

The choice of this plant for the study was based on its numerous nutritional and ethnomedicinal properties. *Moringa* is a cheap and available local source of nutrient for preventing malnutrition among school children (Vanisha *et al,* 2003). According to Ogbuagu *et al,* (2016) *Moringa oleifera* leaf powder has the potential of expelling the adult worm in children infected with soil transmitted helminths with prolongation of the reinfection interval, therefore therapy with *Moringa oleifera* leaf powder can replace deworming with antihelminthic drugs used for school children.

In India, the potentials of *Moringa oleifera* have been exploited in School Supplementary Feeding Programmes (SFP) of the integrated child development scheme as a nutritional supplement which improved their haematological parameters and reduced malarial episodes (Vanisha *et al,* 2003).

This could therefore lead to a simple, cheap and effective solution to the problem of malnutrition and anaemia due to malaria disease in Nigeria. According to Olasehinde *et al* (2012), the potency of crude *M. oleifera* extracts in mice is a confirmation of the rationale for its use in malaria treatment among indigenous Nigerians. Obasi and Mba (2010) also concluded that *Moringa oleifera* leave extract can effectively protect animal hosts against *Plasmodium* by preventing the establishment of new infections and progression of the disease into heavy attacks. Ugwu *et al*, (2013) showed that *Moringa oleifera* leaf extract boosted red blood cell counts in rats and repaired the damages caused by merozoites to the red blood cell in mice that were infected with malaria. Also ethanol leaf extract of *Moringa oleifera* showed significant increase in

haemoglobin concentration in mice when compared with a control group without *Moringa oleifera* (Ugwu *et al*, 2013). All these studies were carried out in animal models in Nigeria. There is therefore need to assess the nutritional and antimalarial effects of *Moringa oleifera* leaf on human subjects.

1.4 Aim and Objectives

The study was aimed at evaluating the nutritional and antimalarial effects of *Moringa oleifera* leaf powder in children aged 4-5 years in Oba community, Idemili- South Local Government Area, Anambra State, Southeast Nigeria.

The Specific Objectives were to determine:

1. The baseline prevalence and intensity of malaria parasite infection among children aged 4 to 5 years in Oba community.
2. The frequency of malaria parasite reinfection among children fed with *Moringa oleifera* leaf powder and children not fed with *Moringa oleifera* leaf powder.
3. The effect of *Moringa oleifera leaf* powder on the nutritional status of the children.
4. The effect of *Moringa oleifera* leaf powder on the haematological parameters such as packed cell volume, haemoglobin, white blood cell count, iron and protein status of the children.

CHAPTER TWO

LITERATURE REVIEW

Malaria is a major cause of illness and death especially among children under 5years old and pregnant women (Nwaorgu and Orajaka, 2011). Between the year 2000 and 2012, estimated malaria mortality rates fell by 42% in all age groups and by 48% in children under 5 years of age (World malaria Report, 2013). Most of the 1-3 million people who die each year from malaria are children, mainly in Africa, which is hyperendemic for malaria. In older children, malaria has a similar course as in adults. However, in children below the age of 5 years, particularly infants, the disease tends to be atypical and more severe. In endemic and hyperendemic areas, the parasite rate increases with age from 0 to 10% during first three months of life to 80 to 90% by one year of age and the rate persists at a high level during early childhood before five years old. The mortality rate is highest during the first two years of life. By school age, a considerable degree of immunity would have developed and asymptomatic parasitaemia can be as high as 75% in primary school children.

2.1 Epidemiology of Malaria

Malaria is an internationally devastating disease, producing nearly 600 million new infections and 3 million deaths each year. The burden of this disease falls heaviest among children below the age of five in sub-Saharan Africa. Nearly 30% of the annual mortality in this population is attributable to malaria. Malaria is caused by protozoan parasites of the genus *Plasmodium*. Four species namely *Plasmdium falciparum, Plasmodium ovale, Plasmodium malariae* and *Plasmodium vivax* affect man. *Plasmodium falciparum* is widespread in Africa while *P. vivax, P. ovale,* and *P. malariae* infections are less common and geographically restricted (Howes *et al*, 2015; Roucher *et al,* 2014). The parasites are transmitted through the bites of infected female *Anopheles* mosquitoes, with *Anopheles gambiae sensu stricto*, *Anopheles funestus* and *Anopheles arabiensis* being the most prevalent in Africa (Sinka *et al*, 2010). Patients present with nonspecific symptoms, including fever, rigors, and chills, and the majority will not require hospital admission. Severe malaria develops in a minority and in children it may manifest as a fever, impaired consciousness, severe anaemia, respiratory distress, convulsions and hypoglycaemia, among other symptoms (Marsh *et al*, 1995). The epidemiology of malaria varies geographically depending on the local malaria transmission intensity or endemicity class (Snow

and Marsh, 2002). While the exact numbers may be uncertain and under-reporting is inevitable, 395 000 deaths were estimated in Africa in 2015 (WHO, 2015). Infection with *P. falciparum* in the absence of overt clinical symptoms is also common (Lindblade *et al*, 2013). It is often referred to as being asymptomatic, but may be better termed chronic and is probably not as benign as the former term might suggest (Chen *et al*, 2016). The epidemiology of malaria can be viewed in terms of being stable (endemic) or unstable (epidemic). Stable malaria refers to a situation in which there is a measurable incidence of natural transmission over several years. This would also include areas which experience seasonal transmission. Persons living in highly endemic areas usually exhibit a high level of immunity and tolerate the infection well. These give an indication of the burden of malaria in a given locale.

Malaria endemicity, as proposed in the WHO (1951) Report on the Malaria Conference in Equatorial Africa can be classified based on spleen rate as follows:

- Hypoendemic malaria: Spleen-rate in children 2–10 years of age, 0–10%.
- Mesoendemic malaria: Spleen-rate in children 2–10 years of age, 11–50%.
- Hyperendemic malaria: Spleen-rate in children 2–10 years of age, constantly over 50%; spleen-rate in adults also high.
- Holoendemic malaria: Spleen-rate in children 2–10 years of age, constantly over 50%; spleen-rate in adults is low. It is in this type of endemicity that the strongest adult tolerance is found.

Malaria endemicity could be classified based on parasite prevalence in the population as follows:

- Hypoendemic malaria: parasite rate in children 2–10 years of age, 0–10%.
- Mesoendemic malaria: parasite rate in children 2–10 years of age, 10–50%.
- Hyperendemic malaria: parasite rate in children 2–10 years of age, 50–75%.
- Holoendemic malaria: parasite rate in children 2–10 years of age, >75%

In Nigeria, malaria is holoendemic in the rural areas and mesoendemic in the urban areas. In the Savanna belt of the country, the transmission rate is approximately uniform throughout the year. In the far North there is a marked difference between the high transmission rate in the short wet season and low transmission rate in the long dry season (Lucas and Gilles, 1998). Transmission

of malaria is intense and stable in Nigeria because the intensity of attack remains constant throughout the year or from year to year (Nwaorgu and Orajaka, 2011).

Unstable or epidemic malaria refers to an increase in malaria in areas of low endemicity or to outbreaks in areas previously without malaria or among non-immune persons. These outbreaks can usually be attributed to changes in human behaviour or effects on the environment. For example, human migration and resettlement can either introduce malaria into an area or expose a previously non-immune population to endemic transmission. Changes in the ecology caused by natural disasters or public works projects such as building roads can also impact malaria transmission and lead to epidemics.

Different communities experience different malaria and consequently different control and treatment strategies may be necessary. The intricate interactions between host, parasite and vector are the major factors in this epidemiological complexity. For example, as with all vector transmitted diseases, the parasite must be able to establish a chronic infection within the host to maximize the opportunities for transmission. This is especially true in the case of seasonal transmission and in areas of low endemicity. And in general malaria infections are characterized by an initial acute phase followed by a longer relatively asymptomatic chronic phase. This is due in part to the ability of the parasite to avoid complete clearance by the immune system. For example, *P. falciparum* exhibits an antigenic variation that allows it to stay one step ahead of the immune system. In addition, *P. vivax* and *P. ovale* exhibit the hypnozoite stage and are capable of relapses. This allows the parasite to maintain the infection within the human host even after the blood stage of the infection has been cleared. The relative long interval between relapses in some *P. vivax* isolates probably explains its ability to maintain transmission cycles in some temperate climates.

Humans are the only significant reservoir for the parasite and sustained transmission depends upon maintaining a pool of infected individuals and contact between humans and *Anopheline* mosquitoes. Several factors influence the susceptibility of humans to infection. Obviously the immune status of the individual and their prior experience with malaria will influence the course of the infection. Pregnant women, especially during the first pregnancy, are more susceptible to *falciparum* malaria as illustrated by a higher prevalence of infection and higher parasitemias. In addition, certain genetic diseases and polymorphisms have been associated with decrease

infection or disease. The potential of the mosquito to serve as a vector depends on the ability to support sporogony, mosquito abundance and contact with humans, which are all influenced by climatic and ecological factors.

The ability to support sporogony is largely dependent upon species in that not all species of *Anopheles* are susceptible to *Plasmodium* infection. Temperature and mosquito longevity are other key factors affecting the parasite's interaction with the vector. Development of *P. falciparum* requires a minimum temperature of 20°C, whereas the minimum temperature for the other species is 16°C. Temperature also affects the time of development in that the duration of sporogony is substantially shorter at higher temperatures. A shorter duration of sporogony increases the chances that the mosquito will transmit the infection within its lifespan.

2.2 Geographical Distribution of malaria

The distribution of malaria across the globe depends mainly on climatic factors such as temperature, humidity, and rainfalls (CDC, 2013). Malaria is transmitted in tropical and subtropical areas, where *Anopheles* mosquitoes can survive and multiply and malaria parasites can complete their growth cycle in the mosquitoes.

Geographical Distribution of Malaria in Africa

In Africa, malaria is present in both rural and urban areas, though the risk is lower in the larger cities (Keisser *et al*, 2004). Thirty countries in Sub-Saharan Africa account for 90% of global malaria deaths. Nigeria, Democratic Republic of Congo (DRC), Ethiopia, and Uganda account for nearly 50% of the global malaria deaths. Malaria is the second leading cause of death from infectious diseases in Africa, after HIV/AIDS. Almost 1 out of 5 deaths of children under 5 in Africa are due to malaria.

Geographical distribution of Malaria in Nigeria

Malaria represents a substantial public health challenge in Nigeria and is a major cause of morbidity and mortality. The country accounts for up to 25% of malaria burden in sub-Saharan Africa, which is globally the highest burden region for malaria (Federal Republic of Nigeria, 2010). In terms of morbidity, around 110 million of clinically diagnosed cases, 30 percent of health care facilities admission and 60 percent of outpatient visits are attributed to the disease each year (WHO, 2012). Malaria is also responsible for 300,000 childhood deaths and 11% maternal deaths annually (WHO, 2010; Kyu *et al*, 2013). The ministry of health reported in April 2004 that malaria is responsible for one out of ten deaths in pregnant women.

Geographical Distribution of Malaria parasite species

The four human malarial species exhibit an overlapping geographical distribution *P. vivax* and *P. falciparum* are the most commonly encountered species with *P. vivax* being the most widespread geographically. Mixed infections are common in endemic areas. Nearly 85% of cases in Africa are caused by *P. falciparum*, the remaining cases being caused by the other three strains. *P. vivax* is now the most geographically widespread of the human malarias, occurring in much of Asia, Central and South America, the Middle East, where 70–90% of the malaria burden is of this species and the rest due to *P. falciparum* (Rich and Ayala, 2006). *P. malariae* causes sporadic infections in Africa, parts of India, western Pacific and South America, whereas *P. ovale* is restricted to tropical Africa, New Guinea, and the Philippines (Carter and Mendis, 2002). *P. knowlesi* has been reported from South East Asian countries such as Malaysia, Thailand, Viet Nam, Myanmar and Phillippines (Cyrus *et al*, 2009).

Plasmodium falciparum has a global distribution and is responsible for the majority of malaria deaths globally and is the most prevalent species in sub-Saharan Africa.

Plasmodium vivax, is the second most significant species. 80-90% of cases occur in Africa, 40-50% of cases in western pacific and South East Asia, 4-30% in South Asia, South America and rest of the tropics. *P. vivax and Plasmodium ovale* have the added complication of a dormant liver stage, which can be reactivated in the absence of a mosquito bite, leading to clinical symptoms.

P. ovale: 70-90% of cases in most of Asia and South America, 50-60% of cases in South East Asia and Western Pacific, 1-10% in Africa

P. malariae: 8% of cases in parts of Africa, stray cases in Asia

P. knowlesi: A species that infects primates has led to human malaria, but the exact mode of transmission remains unclear. Two to three percent in Africa, it is sporadic in Asia and South America (Cyrus *et al*, 2009).

***Plasmodium falciparum* Distribution in Africa**

Climate, local ecology and active control affect the ability of malaria parasites and their *Anopheline* mosquito vectors to coexist long enough to enable transmission. The frequency of transmission or endemicity depends on the density and infectivity of *Anopheline* vectors. These

features depend on a range of climatic, physical and population characteristics, for example, rainfall, location of human settlements near or at rivers or other mosquito larval breeding sites and the density of human populations in a village. The most significant determinant of the intensity of parasite transmission is climate. Previous studies in Nigeria show that *Plasmodium falciparium* is the predominant species found in the blood of children with malaria (Ogolo *et al*, 2015; Nwaorgu and Orajaka, 2011).

Climate determinants of *P. falciparum* transmission in Africa

The development of both the vector and parasite is temperature dependent. The optimum temperature range for parasite development in the female *Anopheles* (sporogony) is between 25°C and 30°C, and development ceases below 16°C. Intermittent low temperatures delay sporogony, and the period immediately after the infective bite by the mosquito on an infected human host is the most sensitive to drops in temperature. Above 35°C sporogony slows down considerably. Extremely high temperatures are associated with the development of smaller and less fecund adult mosquitoes. Thermal death of mosquitoes occurs at 40°C to 42°C. Numerous studies have demonstrated the association between *Anopheles gambiae sensus lato* (the most important vector of *P. falciparum* in Africa) abundance and rainfall. Without surface water the female *Anopheles* cannot lay eggs.

2.3 Prevalence and Intensity of Malaria

Importantly, the composition of the parasite assemblage as well as the prevalence may significantly vary with time, both seasonally and among years (Bensch *et al*, 2007). A number of parameters, associated with the characteristics of the host and the characteristics of its habitat, may contribute to parasite dynamics (Lachish *et al*, 2011b). The susceptibility to parasitic infection is known to be associated with host age and sex. Prevalence and intensity of infection may initially increase with age as new infections accumulate during life, but parasitaemia may disappear among oldest individuals due to selective parasite-dependent mortality and developing resistance (Wilson *et al,* 2001).

Children between the ages of 5 years and teen-age are coming to the fore as having an elevated risk of uncomplicated malaria. They had more episodes with progressively declining transmission (Farnert *et al*, 2014 and Mawili-Mboumba *et al*, 2013), higher than their younger

counterparts aged below 5 years (Mawili-Mboumba *et al*, 2013; Ursing *et al*, 2014). Peak parasite prevalence in asymptomatic infections has also risen to older children and suggests a slowing of the rate at which the ability to control parasites is acquired (Farnert *et al*, 2014; Ishengoma *et al*, 2013). Control strategies that previously focused on under-fives therefore need to extend to older-age categories.

The incidence of malaria among the under fives across six geographical zones in Nigeria were as follows: South-South 32.7%, South West 36.6%, South East 30.7%, North Central 58.8%, North East 55.3% and North West 33.6% (FMOH, 2001). According to the 2010 NMIS, 42% of children age 6-59 months in Nigeria tested positive for malaria by microscopy with lowest prevalence in the South East Zone (28%) and highest in South West (50%) and North Central (49%). Malaria prevalence was markedly lower in the 2015 NMIS, with 27% of children age 6-59 months testing positive by microscopy with lowest prevalence in South East (14%) and South West (17%) and highest in North West (37%) (National Malaria Elimination Program and ICF International, 2016). The study of Ibekwe *et al* (2009) and Onyiri (2015) showed a high prevalence of malaria in Nigeria. The reports of Nwaorgu and Orajaka (2011) showed high malaria prevalence rates of 71.2% in children 4years old and 55.6% in children 5 years old in Awka North Local Government Area. Mbanugo and Emenalo (2004) recorded a high prevalence rate of 77.4% in Owerri and Ukpai and Ajolu (2001) reported high prevalence of 75% in Owerri and 85.5% in Okigwe. Ogolo *et al,* (2015) and Ahmed *et al,* (2001) recorded low prevalence rates of 10.3% among secondary school students in Oba and 6% in Maiduguri respectively.

Individuals born into areas of stable *P. falciparum* transmission frequently acquire and clear infections without becoming ill, but most will, at some stage in their lives, develop an overt clinical response to infection, often manifested as fever. These clinical events may lead to severe complications, which may resolve naturally, require medical intervention or result in death.

The relation between the frequency of parasite exposure and disease outcome is complex. The speed with which a population acquires functional immunity to the severe consequences of *P. falciparum* infection depends on the frequency of parasite exposure from birth as measured by the intensity of parasite transmission in a given locality (Snow and Marsh, 2002). Where infection is rare the risk of mortality is likely to be directly related to the risk of infection, because acquired functional immunity is unlikely to affect health outcomes. Understanding this

relationship is important for defining the age-specific mortality burdens in Sub-Saharan Africa, an area able to support infection rates ranging from one infection every three years to hundreds of new infections per year (Hay *et al*, 2000).

In addition to the morbidity and mortality directly attributed to *P. falciparum,* other consequential and indirect effects are linked to each step of the infection and disease process. Chronic, subclinical infections cause anaemia or may encourage undernutrition, which in turn may increase susceptibility to severe clinical outcomes of subsequent malarial or other pathogenetic infection. Subtler consequences include behavioural disturbances or cognitive impairment (Holding and Snow, 2001; Holding and Wekulo, 2004). In the absence of measures aimed at reducing the risk of infection, the risks largely depend on extrinsic factors, such as those that determine the speed with which a population develops acquired immunity and the access to effective case management and on intrinsic factors such as host genetics.

2.4 Children and Malaria

Over 40% of the world's children live in malaria-endemic countries. Each year, approximately 300 to 500 million malaria infections lead to over one million deaths, of which over 75% occur in African children < 5 years infected with *Plasmodium falciparum* (Snow, 1999). The rapid spread of resistance to antimalarial drugs, coupled with widespread poverty, weak health infrastructure, and, in some countries, civil unrest, means that mortality from malaria in Africa continues to rise. The tragedy is that the vast majority of these deaths are preventable.

Children are vulnerable to malaria from about 4 months of age, and in highly endemic areas during the peak transmission season, approximately 70% of one-year-olds have malaria parasites in their blood. Fever reduces appetite, and exacerbates malnutrition. Recurrent episodes of malaria in the child, or in a family member may mean that the child is required to stay at home to help with domestic chores, are likely to result in the loss of a substantial amount of time from school.

Approximately 7% of children who survive cerebral malaria (a severe form of the disease, characterized by coma and convulsions) are left with permanent neurological problems. These include weakness, spasticity, blindness, speech problems and epilepsy. The limited availability

of specialized educational provision and equipment for such children means that opportunities for subsequent learning, and for attainment of independence, are compromised even further. Epilepsy may be inadequately treated, or untreated, due to lack of appropriate drugs and expertise, and further injury or death may result from uncontrolled convulsions. Recent evidence suggests that some children who appear to have made a complete neurological recovery from cerebral malaria may develop significant cognitive problems (attention deficits, difficulty with planning and initiating tasks, speech and language problems), which can adversely affect school performance (Holding, 1999).

It has been estimated that severe malarial anaemia causes between 190 000 and 974 000 deaths each year among children < 5 years (Murphy and Breman, 2001). Although blood transfusion may be life-saving in this situation, it also exposes children to the risk of HIV and other blood-borne diseases. It is estimated that African children have between 1.6 and 5.4 episodes of malarial fever each year, a figure that varies according to geographical and epidemiological circumstances (Murphy and Breman, 2001). Issaka *et al*, (2002) noted a 7 to 9 weeks post treatment reinfection with malaria parasite, a delay consistent with the absence of significant drug resistance. Woodring *et al* (2010) observed a 37 days recurrent malaria parasitaemia in children after treatment with artemether-lumefantrine.

Malaria and Undernutrition

It has been postulated that nutritional status is related to the threats posed by infection and disease and to the role of infectious disease in perpetuating under-nutrition (Pelletier *et al*, 1995). Most studies have generally focused on severe malnutrition and specific nutrient deficiency, and only a few have examined the role of malaria. One striking feature of the global distribution of anthropometric markers of undernutrition is its congruence with the distribution of endemic malaria. Although *P. falciparum* malaria and malnutrition are both highly prevalent in Sub-Saharan Africa, the existence of a synergistic interaction has not been well established. Evidence from intervention trials aimed at reducing the frequency of new infections suggests that malaria infection might have some indirect effects upon the generalized nutritional status of African children. A study in Nigeria on the use of chemoprophylaxis in the treatment of malaria in children showed a reduction in the incidence of infection and clinical attacks that was

accompanied by a reduction in the incidence of malnutrition (Bradley-Moore *et al*, 1985). Improved growth among young children has more recently been demonstrated in The Gambia and Kenya in studies comparing those protected by insecticide-treated bednets with those left unprotected (Ter Kuile *et al*, 2003). Despite the biological plausibility of synergism between infection and growth, the precise relationship between undernutrition and severe malaria continues to be difficult to quantify empirically within disease burden frameworks.

2.5 Immunity in Malaria

Persons living in endemic areas do develop immunity against malaria. Almost always a person will exhibit symptoms during their initial exposures to malaria. Symptoms associated with subsequent exposures to malaria are usually less severe, though the immunity against malaria is slow to develop and requires multiple exposures. In highly endemic areas only young children are at a high risk of developing severe *falciparum* malaria whereas older children and adults are essentially protected from severe disease and death (Newton and Krishna, 1998). However, this immunity is not a sterilizing immunity in that persons can still become infected. In addition the immunity is short lived and in the absence of repeated exposure the level of immunity decreases. For example, previously semi-immune adults will often develop severe malaria upon returning to an endemic area after being in a non-endemic area for 1-2 years. This state of partial immunity in which parasitaemia is lowered, but not eliminated, and parasitaemia is better tolerated is sometimes referred to as premunition. Premunition refers to an immunity that is contingent upon the pathogen being present. Malaria infection is common in Sub-Saharan Africa, but death directly attributed to the parasite is comparatively rare, largely because of acquired functional immunity. These deaths are concentrated among those with poorly developed immunity, and, generally, young children bear the brunt of the mortality burden. Many individuals naturally acquire functional immune responses to severe disease and death early in life; immunity to the milder consequences of infection occurs later in childhood, but the ability to sterilize blood-stage infection probably does not occur until adulthood (Murphy and Breman, 2001).

The incubation period is the time between infection and the appearance of symptoms. The immune response could be directed at either the pre-erythrocytic or erythrocytic stages of the parasite's life cycle. However, the erythrocytic stage of the life cycle is probably the most important in terms of clearing the parasite and lessening the disease. Due to the lack of Human

Leukocyte Antigen (HLA) molecules on the surface of the parasite or the erythrocyte it is usually assumed that antibody will play a key role in blood-stage immunity. Possible effector mechanisms for antibody include: blocking erythrocyte invasion by merozoites, antibody-dependent cellular killing mediated by cytophilic antibodies, or increased clearance of infected erythrocytes due to binding of antibodies to parasite antigens exposed on the erythrocyte surface. All of these will result in lower parasitaemia. The relative importance of these various mechanisms is not clear and probably immunity probably requires the generation of antibodies against numerous targets. This, along with antigenic variation and polymorphisms in many *Plasmodium* antigens, could explain the slow development of immunity(Murphy and Breman, 2001).

2.5.1 Human Genetics and Innate Resistance

Certain genetic diseases and polymorphisms have been associated with decreased infection or disease. Several inherited erythrocyte disorders are found predominantly in malaria endemic areas and at frequencies much higher than expected. This has led to speculation that these disorders confer some protection against malaria. In most cases it is presumed or speculated that the combination of the defects and infection leads to premature lysis or clearance of the infected erythrocyte (Williams, 2006).

Types of Innate Resistance

Evidence has accumulated that the first line of defense against malaria is provided by genetically controlled innate resistance, mainly exerted by abnormal haemoglobins and glucose-6-phosphate dehydrogenase deficiency. The three major types of inherited genetic resistance sickle cell disease, thalassemias and G6PD deficiency were present in the Mediterranean world by the time of the Roman Empire.

a. Sickle-Cell Disease

The most-studied influence of the malaria parasite upon the human genome is a hereditary blood disease, sickle-cell disease. The sickle-cell trait causes disease, but even those only partially affected by sickle cell have substantial protection against malaria. Sickle cell anaemia is speculated to make the infected erythrocyte more susceptible to oxidative stress (Min-Oo and Gros, 2005). In sickle-cell disease, there is a mutation in the *HBB* gene, which encodes the beta-globin subunit of haemoglobin. The normal allele encodes a glutamate at position six of the beta-

globin protein, whereas the sickle-cell allele encodes a valine. This change from a hydrophilic to a hydrophobic amino acid encourages binding between haemoglobin molecules, with polymerization of haemoglobin deforming red blood cells into a "sickle" shape. Such deformed cells are cleared rapidly from the blood, mainly in the spleen, for destruction and recycling.

In the merozoite stage of its life cycle, the malaria parasite lives inside red blood cells, and its metabolism changes the internal chemistry of the red blood cell. Infected cells normally survive until the parasite reproduces, but, if the red cell contains a mixture of sickle and normal haemoglobin, it is likely to become deformed and be destroyed before the daughter parasites emerge. Thus, individuals heterozygous for the mutated allele, known as sickle-cell trait, may have a low and usually unimportant level of anaemia, but also have a greatly reduced chance of serious malaria infection. This is a classic example of heterozygote advantage. However, in populations where malaria is endemic, the frequency of sickle-cell genes is around 10%. There are also other mutations of the HBB gene that produce haemoglobin molecules capable of conferring similar resistance to malaria infection. These mutations produce haemoglobin types HbE and HbC, which are common in Southeast Asia and Western Africa, respectively.

b.Thalassaemias

Another well-documented set of mutations found in the human genome associated with malaria are those involved in causing blood disorders known as thalassaemias. Studies in Sardinia and Papua New Guinea have found that the gene frequency of β-thalassaemias is related to the level of malarial endemicity in a given population (Min-Oo and Gros, 2005). A study on more than 500 children in Liberia found that those with β-thalassaemia had a 50% decreased chance of getting clinical malaria. Similar studies have found links between gene frequency and malaria endemicity in the α+ form of α-thalassaemia. Thalassemia is speculated to make the infected erythrocyte more susceptible to oxidative stress (Min-Oo and Gros, 2005).

c. Glucose 6 phosphate dehydrogenase (G6PD)

Glucose-6-phosphate dehydrogenase (G6PD) is an enzyme that normally protects from the effects of oxidative stress in red blood cells. However, a genetic deficiency in this enzyme results in increased protection against severe malaria. For example, glucose-6-phosphate dehydrogenase (G6PD) deficient erythrocytes would have an impaired ability to handle oxidative stress. The

additional oxidants produced as a result of parasite metabolism and the digestion of haemoglobin may overwhelm the infected erythrocyte and lead to its destruction before the parasite is able to complete schizogony.

d. Duffy Antigens

The Duffy antigens are antigens expressed on red blood cells and other cells in the body acting as a chemokine receptor. The expression of Duffy antigens on blood cells is encoded by Fy genes (Fya, Fyb, Fyc etc.). *Plasmodium vivax* malaria uses the Duffy antigen to enter blood cells. However, it is possible to express no Duffy antigen on red blood cells (Fy-/Fy-). This genotype confers complete resistance to *P. vivax* infection (Carter and Mendis, 2002). For example, individuals who lack the Duffy blood-group antigen are refractory to *P. vivax*. A large proportion of the populations in western Africa are Duffy negative, thus accounting for the low levels of *P. vivax* in West Africa. This innate resistance led to the identification of the Duffy antigen as the erythrocyte receptor for merozoite invasion. The genotype is very rare in European, Asian and American populations, but is found in almost all of the indigenous population of West and Central Africa. This is thought to be due to very high exposure to *P. vivax* in Africa in the last few thousand years (Carter and Mendis, 2002).

e. Southeast Asian Ovalcytosis (SAO)

Ovalocytosis a subtype of elliptocytosis is an inherited condition in which erythrocytes have an oval instead of a round shape. In most populations ovalocytosis is rare, but South-East Asian ovalocytosis (SAO) occurs in as many as 15% of the indigenous people of Malaysia and of Papua New Guinea. This is due to a mutation in an erythrocyte membrane protein called band 3. These qualitative defects create a red blood cell membrane that is less tolerant of shear stress and more susceptible to permanent deformation. SAO is associated with protection against cerebral malaria in children because it reduces sequestration of erythrocytes parasitized by *P. falciparum* in the brain microvasculature (Allen *et al*, 1999). Adhesion of *P. falciparum*-infected red blood cells to CD36 is enhanced by the cerebral malaria-protective SAO trait. Higher efficiency of sequestration via CD36 in SAO individuals could determine a different organ distribution of sequestered infected red blood cells. These provide a possible explanation for the selective advantage conferred by SAO against cerebral malaria (Cortés *et al*, 2005). This mutation causes

the erythrocyte membrane to become more rigid and more refractory to merozoite invasion (Williams, 2006).

f. Human Leucocyte Antigen (HLA and Interleukin-4)

Human leucocyte antigen (HLA) polymorphisms common in West Africans but rare in other racial groups, are associated with protection from severe malaria. Human leucocyte Antigen (HLA)-B53 is associated with low risk of severe malaria. This Major Histocompatibility (MHC) class I molecule presents liver stage and sporozoite antigens to T-Cells. Interleukin-4, encoded by IL4, is produced by activated T cells and promotes proliferation and differentiation of antibody-producing B cells. A study of the Fulani of Burkina Faso, who have both fewer malaria attacks and higher levels of antimalarial antibodies than do neighboring ethnic groups, found that the IL4-524 T allele was associated with elevated antibody levels against malaria antigens, which raises the possibility that this might be a factor in increased resistance to malaria (Verra *et al*, 2004).

2.5.2 Acquired /Adaptive immunity in Maiaria

In humans, various types of acquired or adaptive immunity against *Plasmodia* have been defined: (i) antidisease immunity, conferring protection against clinical disease, which affects the risk and extent of morbidity associated with a given parasite density; (ii) antiparasite immunity, conferring protection against parasitemia, which affects the density of parasites; and (iii) premunition, providing protection against new infections by maintaining a low-grade and generally asymptomatic parasitemia (Sergent and Parrot, 1935).

Across sub-Saharan Africa where the disease is holoendemic, most people are almost continuously infected by *P. falciparum*, and the majority of infected adults rarely experience overt disease. They go about their daily routines of school, work, and household chores feeling essentially healthy despite a population of parasites in their blood that would almost universally prove lethal to a malaria-naive visitor. This vigor in the face of infection is natural acquired immunity to *P. falciparum* malaria. Adults have natural acquired immunity, but infants and young children, at least occasionally, do not. Natural Acquired Immunity is compromised in pregnant women, especially primigravidae. Adults removed from their routine infections apparently lose natural acquired immunity, at least temporarily. Interventions that reduce

exposure below a level capable of maintaining natural acquired immunity risk the possibility of catastrophic rebound, as occurred in the highlands of Madagascar in the 1980s, with epidemic malaria killing more than 40,000 people (Romi *et al*, 2002). Routine exposure to hyper- to holoendemic malaria protects a majority of individuals while killing a minority.

2.6 Pathophysiology of Malaria

Malaria in humans develops via two phases: an exoerythrocytic and an erythrocytic phase. The exoerythrocytic phase involves infection of the hepatic system, or liver, whereas the erythrocytic phase involves infection of the erythrocytes, or red blood cells.

Pre-erythrocytic schizogony: Sporozoites are injected by the mosquito into the subcutaneous tissue (less frequently directly into the bloodstream) and travel to the liver either directly or through lymphatic channels. They reach the liver in 30-40 minutes by brisk motility conferred by Circum Sporozoite Protein (CSP). Approximately 8-15 (up to 100) sporozoites are injected and therefore only a few hepatocytes are infected, therefore this stage of the infection causes no symptoms (rarely a prodromal illness characterized by vague aches and pains, headache, nausea etc. may be present). Recent evidence indicates that sporozoites pass through several hepatocytes before invasion. The co-receptor on sporozoites for invasion involves, in part, the thrombospondin domains on the circumsporozoite protein and on thrombospondin-related adhesive protein (TRAP). These domains bind specifically to heparin sulfate proteoglycans on hepatocytes in the region in opposition to sinusoidal endothelium and Kuppfer cells. Within the hepatocyte, each sporozoite divides into 10000-30000 merozoites. This phase is called pre-erythrocytic schizogony, meaning development of schizoid forms of the parasite before reaching the red blood cells. This phase takes about 10 - 15 days in *P. vivax* malaria and about 7-10 days in *P. falciparum* malaria.

Erythrocytic schizogony: At the completion of the pre-erythrocytic schizogony, the mature schizonts rupture the liver cells and escape into the blood, wherein they infect the red blood cells. These infective forms are called merozoites and they continue their growth and multiplication within the red blood cells. In *P. vivax* malaria, the young red blood cells are predominantly infected, while in *P. falciparum* malaria, red blood cells of all ages are affected. Thus the infective load and severity of infection are more in case of *P. falciparum* malaria.

The sequence of invasion is probably similar for all *Plasmodium* spp. The merozoite first attaches to red cells. In *P. falciparum*, Erythrocyte Binding Antigen 175 and Merozoite Surface

Protein 1, 2 with sialoglycoproteins have been identified as the ligands and in *P. vivax*, Duffy antigen on red blood cells is the site of binding. After the attachment to the red cell, the merozoite reorientates itself so that apposition of apical end occurs. This is followed by localized invagination and interiorization of the merozoite. The entire process is completed in 30 seconds. The growth and multiplication cycle within the red blood cells (*Erythrocytic schizogony*) takes about 48 hours for one cycle (72 hours in case of *P. malariae*). Each merozoite divides into 8-32 (average 10) fresh merozoites. The merozoites grow in stages into rings - trophozoites and divide in a Schizont to release more merozoites. At the end of this cycle, the mature schizonts rupture the red blood cells and release the new merozoites into the blood, which in turn infect more red blood cells. The parasite has complex metabolic processes: It utilizes amino acids from haemoglobin and detoxifies heme; pLDH, *Plasmodium* aldolase have been identified as enzymes of anaerobic glycolysis. It has been found that the parasites increase the permeability of red blood cells to get nutrients, yet maintain the red blood cells structure for 48 hours. At the end, red blood cells ruptures and each schizont releases 6-36 merozoites.

Merozoite's Nutrition in red blood cells: The parasite ingests hemoglobin from red blood cells to form a food vacuole where it is degraded and heme is released. The toxic heme is in turn detoxified by heme polymerase and sequestrated as hemozoin (malaria pigment). All the clinical features of malaria are caused by these developments in the blood. The growing parasite progressively consumes and degrades intracellular proteins, principally haemoglobin, resulting in formation of the 'malarial pigment' and hemolysis of the infected red cell. This also alters the transport properties of the red cell membrane, and the red cell becomes more spherical and less deformable. The rupture of red blood cells by merozoites releases certain factors and toxins (such as lycosylphosphotidylinositol anchor of a parasite membrane protein, phospholipoprotein, red blood cells membrane products, protease sensitive components with hemozoin, malarial toxins etc.), which could directly induce the release of cytokines such as Tumour necrotic factor (TNF) and interleukin-1 from macrophages, resulting in chills and high grade fever. This occurs once in 48 hours, corresponding to the erythrocytic cycle.

In the initial stages of the illness, this classical pattern may not be seen because there could be multiple groups (broods) of the parasite developing at different times, and as the disease progresses, these broods join and the synchronous development cycle results in the classical pattern of alternate day fever. It has been observed that in primary attack of malaria, the

symptoms may appear with lesser degree of parasitemia or even with submicroscopic parasitemia.

However, in subsequent attacks and relapses, a much higher degree of parasitemia is needed for onset of symptoms. Further, there may be great individual variations with regard to the degree of parasitemia required to induce the symptoms in different malaria species. The progeny from a single parasite in the liver could destroy all the host's red blood cells within 12- 14 days.

In case of *P. vivax* infection, the parasitemia is limited by exhaustion of suitable red cells, specific and non-specific immune response and destruction of meronts by high fevers and splenic clearance.

In *P. falciparum* infection, the available red cell numbers are higher as red cells of all ages are infected and the parasitized red cells escape from destruction by sequestration. The *P. falciparum* has better skills for attack and can enter most red blood cells, has plenty of redundant receptors and pathways and hence higher parasitemia. It also has better skills for survival: the parasite constantly changes itself with 2% antigenic variation, it changes the red blood cells structure by producing sticky Knobs on the surface, and it changes the immune response by numbing the dendritic cells and hides in deeper tissues by sequestration. *P. falciparum* malaria is characterized by development of sticky knobs on the surface of red cells, adhesion of red cells to the endothelial cells of post-capillary venules and formation of *rosettes* with uninfected cells.

Rosetting is adherence of parasitized red cells with uninfected red cells. It is independent of venular cytoadherence and exhibits 5 times stronger adhesion than cytoadherence. Rosetting causes higher microvascular obstruction than cytoadherence and is associated with cerebral malaria (cytoadherence with other vital organ damage). Rosetting reduces blood flow, encourages cytoadherence to endothelium, enhances anaerobic glycolysis and reduces the pH.

As the parasite matures, flexible biconcave disc becomes progressively more spherical and rigid. Reduced membrane fluidity, increasing sphericity, enlarging and relatively rigid intra-erythrocytic parasites make the red cells less filterable and cause obstruction at mid capillary level itself. Unbridled *cytoadherence-rosetting-sequestration* results in poor tissue perfusion, organ dysfunction, anaerobic glycolysis in tissues and lactic acidosis, malfunctioning of dendritic cells and T cells due to CD36 binding. While low levels of cytokines may be beneficial, high

levels are found to be harmful, contributing to placental dysfunction, suppression of erythropoeisis, inhibition of gluconeogenesis and increased cytoadherence.

Exo-erythrocytic schizogony

In *P. vivax* and *P. ovale,* some exo-erythrocytic forms remain as single celled dormant forms called *hypnozoites*. This helps them to survive in temperate countries. These hypnozoites can get re-activated once in 3-6 months to cause 'relapses'. This phase of the infection is called an *exo-erythrocytic schizogony.* In *P. falciparum* and *P. malariae* infections, relapses from the liver do not occur; however, the blood infection may remain chronic and, if untreated, may remain for years in case of *P. falciparum* and decades in case of *P. malariae*.

Some of the merozoites in the blood transform into sexual forms, known as gametocytes. These appear in the peripheral blood after 7-10 days of the infection in *P. vivax* and 10-20 days in *P. falciparum* infection. When anopheles mosquito bites an infected individual, these gametocytes enter the mosquito and continue their sexual phase of development within the gut wall of the mosquito. This completes the asexual - sexual cycle of the malaria parasite.

Direct Malaria Mortality

Malaria is often difficult to diagnose on a purely clinical basis. Fever is common to almost every infectious disease, and the severe pathology caused by *P. falciparum,* such as acidosis, anaemia and altered consciousness are also complications of other infections. When a person is ill, demonstrating the presence of malaria infection increases the likelihood that symptoms are directly due to the infection, but the high prevalence of asymptomatic infections makes it difficult to exclude other diagnoses. Our earlier understanding of the pathophysiology of malaria derived from clinical descriptions among adults in Southeast Asia and only recently have the mechanisms of death been more precisely defined for pediatric African populations (Marsh *et al,* 1995). Several detailed clinical studies in African hospital settings have described the principal, sometimes overlapping routes to a fatal outcome. These include cerebral involvement from sequestered infection in the vasculature of the brain, metabolic disturbances, respiratory distress and severe anemia. For epidemiological purposes it is convenient to define two major syndromes, cerebral malaria (CM) and severe malarial anaemia (SMA). CM is a condition in which patients present in coma with several underlying causes, ranging from a primarily neurological condition to a systemic metabolic disturbance (Marsh and Snow 1999; Newton and

Krishna 1998). Severe anaemia is pathology of life-threatening malaria with a complex etiology combining rapid haemolysis during acute infection or a slow insidious process compounded by antimalarial drug resistance. SMA is a life-threatening condition in young children and often warrants blood transfusion.

Overall malaria-specific mortality in children is approximately 3.5 times higher in areas of stable endemic transmission than in areas of low intensity, stable, or epidemic-prone malaria in Sub-Saharan Africa, excluding southern Africa. Mortality declines rapidly with increasing age, and this is especially striking under conditions of stable endemic transmission. The mortality rates for all ages from *P. falciparum* in southern Africa are considerably lower than those described for the rest of Africa, reflecting a low risk of infection combined with effective control.

Neurological disability associated with severe malaria

The case-fatality rate of cerebral malaria in most hospital settings is high, often over 30 percent (Newton and Krishna, 1998). Prolonged coma and seizures are associated with neurological impairment in survivors. The immediate and prolonged sequelae associated with cerebral malaria among African children include hemiparesis, quadriparesis or severe deficit, hearing and visual impairments, speech and language and nonverbal construction difficulties, behavioral problems, and epilepsy (Mung'ala-Odera *et al*, 2004). These impairments are estimated to occur in about 4,000 children each year in Sub-Saharan Africa. Those with severe deficits have a higher mortality risk soon after the disease event. The risk of premature mortality could be as high as nine times that described in age-comparable groups without epilepsy (Coleman *et al*, 2002). This is likely to be caused by poorly managed epilepsy resulting in status epilepticus or accidents, such as drowning or burns.

2.7 Diagnosis of Malaria

Malaria is suspected in persons with a history of being in an endemic area and presenting symptoms consistent with malaria. These symptoms, especially in the early stages of the infection, are non-specific and often described as flu-like. As the disease progresses, the patient may exhibit an enlarged spleen or liver and anemia. Diagnosis is confirmed by microscopy. Recent investigations suggest that malarial retinopathy is better (collective sensitivity of 95% and specificity of 90%) than any other clinical or laboratory feature in distinguishing malarial from non-malarial coma (Beare *et al*, 2006). Diagnosing malaria infection with *Plasmodium*

parasites may or may not lead to symptoms recognizable as an acute infection prompting care. The following methods may be used in the diagnosis of malaria.

- Presumptive or Clinical Diagnosis

For decades, presentation with a fever, particularly in children, was assumed to be malaria and treated with antimalarials. This practice continues when diagnostic tools are not available (e.g., under resourced clinics, retail outlets). Although the presence of fever is highly sensitive for malaria, it lacks specificity which leads to over diagnosis. These cases are counted as malaria cases although they may be designated 'unconfirmed' or 'clinical' malaria.

- Diagnosis by Light Microscopy

Examination of stained blood smears by light microscopy has been used to detect parasites in the peripheral blood since the late 1800s. Microscopy is inexpensive and, when done well, can be very sensitive and specific. The quality of microscopic diagnosis is often compromised by poor equipment, under qualified staff, high workload and limited availability of electricity and reagents.

- Diagnosis by Rapid Diagnostic Test (RDT)

Immunochromatographic antigen-based single-use tests detect circulating antigen in an active infection. Tests can have excellent sensitivity and specificity compared to light microscopy, with some loss of sensitivity at very low parasite densities. Tests based on the HRP2 antigen do exhibit reduced specificity since HRP2 can circulate in the bloodstream for a week after treatment. RDTs are simple, do not require electricity, and their availability has greatly expanded the reach of diagnosis. New technologies, including urine and saliva tests, are under development. Highly sensitive PCR methods are used primarily for research. However, the recently developed loop-mediated isothermal amplification (LAMP), a nucleic acid-based diagnostic tool that is cheaper, faster, and easier than PCR but with equal sensitivity, has potential for application in targeting the malaria infectious reservoir through mass screening and treatment (MSAT). In Africa, case numbers obtained through routine health information systems are confounded by two main problems: (i) incomplete reporting (low reporting from the public sector, and lack of engagement of private sector in government reporting), and (ii) inclusion of both unconfirmed cases and diagnostically confirmed cases in the totals. Because clinical

diagnosis has very low specificity, when diagnosis is scaled up the total malaria case numbers may be observed to decline.

Examination of Blood Films

The most economic, preferred, and reliable diagnosis of malaria is microscopic examination of blood films because each of the four major parasite species has distinguishing characteristics. Two types of blood film are traditionally used. Thin films are similar to usual blood films and allow species identification because the parasite's appearance is best preserved in this preparation. Thick films allow the microscopist to screen a larger volume of blood and are about eleven times more sensitive than the thin film, so picking up low levels of infection is easier on the thick film, but the appearance of the parasite is much more distorted and therefore distinguishing between the different species can be much more difficult. With the pros and cons of both thick and thin smears taken into consideration, it is imperative to utilize both smears while attempting to make a definitive diagnosis (Warhurst and Williams, 1996). From the thick film, an experienced microscopist can detect parasite levels (or parasitemia) down to as low as 0.0000001% of red blood cells. Diagnosis of species can be difficult because the early trophozoites ("ring form") of all four species look identical and it is never possible to diagnose species on the basis of a single ring form; species identification is always based on several trophozoites.

Field Tests

In areas where microscopy is not available, or where laboratory staff are not experienced at malaria diagnosis, there are antigen detection tests that require only a drop of blood (Pattanasin *et al,* 2003). Immunochromatographic tests (also called: Malaria Rapid Diagnostic Tests, Antigen-Capture Assay or "Dipsticks") have been developed, distributed and field-tested. These tests use finger-stick or venous blood, the completed test takes a total of 15–20 minutes, and a laboratory is not needed. The threshold of detection by these rapid diagnostic tests is in the range of 100 parasites/µl of blood compared to 5 by thick film microscopy.

The first rapid diagnostic tests were using *P. falciparum* glutamate dehydrogenase as antigen (Ling *et al,* 1986). PGluDH was soon replaced by *P. falciparum* lactate dehydrogenase (PLDH),

a 33-kDa oxidoreductase (EC 1.1.1.27). It is the last enzyme of the glycolytic pathway, essential for ATP generation and one of the most abundant enzymes expressed by *P.falciparum*. PLDH does not persist in the blood but clears about the same time as the parasites following successful treatment. The lack of antigen persistence after treatment makes the pLDH test useful in predicting treatment failure. In this respect, pLDH is similar to pGluDH. The OptiMAL-IT assay can distinguish between *P. falciparum* and *P. vivax* because of antigenic differences between their pLDH isoenzymes. OptiMAL-IT will reliably detect *falciparum* down to 0.01% parasitemia and non-*falciparum* down to 0.1%. *Para*check-Pf will detect parasitemias down to 0.002% but will not distinguish between *falciparum* and non-*falciparum* malaria. Parasite nucleic acids are detected using polymerase chain reaction. This technique is more accurate than microscopy. However, it is expensive, and requires a specialized laboratory. Moreover, levels of parasitemia are not necessarily correlative with the progression of disease, particularly when the parasite is able to adhere to blood vessel walls. Therefore more sensitive, low-tech diagnostic tools need to be developed in order to detect low levels of parasitaemia in the field. Areas that cannot afford even simple laboratory diagnostic tests often use only a history of subjective fever as the indication to treat for malaria. Using Giemsa-stained blood smears from children in Malawi, one study showed that unnecessary treatment for malaria was significantly decreased when clinical predictors (rectal temperature, nail bed pallor, and splenomegaly) were used as treatment indications, rather than the current national policy of using only a history of subjective fevers (sensitivity increased from 21% to 41%) (Redd *et al,* 2006).

Molecular Methods

Molecular methods are available in some clinical laboratories and rapid real-time assays (for example, QT-NASBA based on the polymerase chain reaction) (Mens *et al*, 2006) are being developed with the hope of being able to deploy them in endemic areas.

2.8 Treatment of Malaria

In endemic areas, the World Health Organization recommends that treatment be started within 24 hours after the first symptoms appear. Treatment of patients with uncomplicated malaria can be conducted without hospitalization, but patients with severe malaria should be hospitalized if possible. Active malaria infection with *P. falciparum* is a medical emergency requiring

hospitalization. Infection with *P. vivax*, *P. ovale* or *P. malariae* can often be treated on an outpatient basis. Treatment of malaria involves supportive measures as well as specific antimalarial drugs. The fixed-dose combination, artemether-lumefantrine (AL), and the copackaged combination of amodiaquine and artesunate (AQ + AS) is the treatment regime adopted in 2004 (WHO, 2015 ; Kar *et al*, 2014). To date, 28 countries have adopted AL as first-line treatment of uncomplicated malaria (Novartis, 2007). When properly treated, someone with malaria can expect a complete recovery (CDC, 2006). Broadly speaking, anti-malaria drugs can be divided into four major classes viz: Blood Schizonticides, Antifolates, Antimitochondrials and Redox Process-Based Agents.

- **Blood schizonticides**

When the malaria parasite leaves the liver and penetrates an erythrocyte, it can at last begin a haemoglobin diet. However, it cannot use the iron-containing haem group. Released ferriprotoporfyrin IX is toxic for the parasite, it contains trivalent iron (ferric = Fe^{3+}). Normally the parasite polymerises haemin to non-toxic malaria pigment. Chloroquine, quinine, mefloquine and halofantrine interfere with the detoxification of haemin in the digestive vacuole of the parasite. The drugs prevent this detoxification so that haemin can generate free radicals and membrane damage follows. It is therefore logical that the drugs are not active against the parasitic stages which precede the blood forms (sporozoites, liver forms) and which do not consume haemoglobin.

- **Antifolates**

Folic acid is an important metabolic factor. Humans obtain this vitamin from the food they eat. The malaria parasite, on the other hand, must produce it for itself. Para-aminobenzoic acid (PABA) is used at an early stage of the biosynthesis of folic acid by the enzyme dihydropteroate synthetase. This step is inhibited by structural analogues of PABA, such as sulphonamides and sulphones, e.g. sulphanilamide, sulphadoxine and dapsone. The next synthesis step is catalyzed by dihydrofolate reductase. This step is prevented by pyrimethamine, trimethoprim and cycloguanil (proguanil); to such an extent that tetrahydrofolate the end product is not formed.

- **Antimitochondrials**

Although artemisinin derivatives and 8-aminoquinolines cause mitochondrial swelling, this organelle is not their chief target. Some antibiotics such as tetracycline and clindamycin prevent protein synthesis by mitochondrial ribosomes (these are similar to the ribosomes found in bacteria). They are slow-acting. Atovaquone is a naphthoquinone which specifically destroys the electron transport chains of Apicomplexa. The molecule is rather similar to ubiquinone (coenzyme Q) which plays a role in the energy transfer between cytochrome B and C1. The enzymes of *Plasmodium falciparum* are 1000 times more sensitive to atovaquone than the corresponding enzymes in humans. Resistance can easily develop if it is used in monotherapy.

- **Redox reactions**

Primaquine and Etaquine exercise their action via redox-active quinone metabolites. They are selectively toxic for the pre-erythrocytic stages and are the only medicaments which kill hypnozoites. Etaquine has in addition a pronounced blood schizonticidal action.

2.9 Prevention and Control of Malaria

Methods used to prevent the spread of disease or to protect individuals in areas where malaria is endemic include prophylactic drugs, mosquito eradication, and the prevention of mosquito bites. The continued existence of malaria in an area requires a combination of high human population density, high mosquito population density, and high rates of transmission from humans to mosquitoes and from mosquitoes to humans. If any of these is lowered sufficiently, the parasite will sooner or later disappear from that area, as happened in North America, Europe and much of Middle East. However, unless the parasite is eliminated from the whole world, it could become re-established if conditions revert to a combination that favors the parasite's reproduction. Many countries are seeing an increasing number of imported malaria cases due to extensive travel and migration.

Brazil, Eritrea, India, and Vietnam have, unlike many other developing nations, successfully reduced the malaria burden. Common success factors included conducive country conditions, a targeted technical approach using a package of effective tools, data-driven decision-making, active leadership at all levels of government, involvement of communities, decentralized

implementation and control of finances, skilled technical and managerial capacity at national and sub-national levels, hands-on technical and programmatic support from partner agencies, and sufficient and flexible financing (Barat, 2006).

Vector control

Before DDT, malaria was successfully eradicated or controlled also in several tropical areas by removing or poisoning the breeding grounds of the mosquitoes or the aquatic habitats of the larva stages, for example by filling or applying oil to places with standing water. These methods have seen little application in Africa for more than half a century (Killeen *et al,* 2002).

On December 21, 2007, a study published in PLoS Pathogens found that the hemolytic C-type lectin CEL-III from *Cucumaria echinata*, a sea cucumber found in the Bay of Bengal, impaired the development of the malaria parasite when produced by transgenic mosquitoes (Yoshida *et al*, 2007). This could potentially be used one day to control malaria by using genetically modified mosquitoes refractory to the parasites, although the authors of the study recognize that there are numerous scientific and ethical problems to be overcome before such a control strategy could be implemented. Studies in Nigeria also show that *Moringa oleifera* seed oil has a toxic action against the larvae of *A.aegypti* (Nwankwo *et al,* 2010).

- **Prophylactic drugs: Malaria prophylaxis**

Several drugs, most of which are also used for treatment of malaria can be taken preventively. Generally, these drugs are taken daily or weekly, at a lower dose than would be used for treatment of a person who had actually contracted the disease. Use of prophylactic drugs is seldom practical for full-time residents of malaria-endemic areas, and their use is usually restricted to short-term visitors and travelers to malarial regions.

Chemoprophylaxis requires the use of non-toxic drugs since these drugs will be taken over extended periods of time. Generally the patient will start to take the drug before traveling and then continue taking the drug during the stay in the endemic area and continue taking the drug after returning. This is to ensure that the drug is maintained at sufficient levels throughout the visit and to protect against any infection obtained during the visit. Unfortunately, many of the effective and non-toxic drugs (e.g., chloroquine, pyrimethamine, proguanil) are of limited use

because of drug resistance. Another strategy is presumptive (or 'standby') treatment to be used in conjunction with prophylaxis. In this case a person either forgoes prophylaxis or takes chloroquine or another relatively non-toxic drug for prophylaxis and carries a drug like Fansidar, mefloquine, or quinine, which they will take if they start to exhibit symptoms associated with malaria (Newton and White 1999).

Mefloquine is efficacious at preventing malaria with a single dose per week, thus offering advantages to drugs that need to be administered daily. At this dosage mefloquine is tolerated by most individuals. However, some people experience neuropsychiatric adverse affects such as sleep disturbances and nightmares. Killing the exoerythrocytic stage (i.e., liver) would prevent the blood infection and is known as causal prophylaxis. This is highly desirable in that it limits the amount of time the prophylactic drug needs to be taken before and after travel to an endemic area. The only currently available drug for causal prophylaxis is primaquine.

- **Indoor residual spraying**

Indoors residual spraying (IRS) is the practice of spraying insecticides on the interior walls of homes in malaria-affected areas. After feeding, many mosquito species rest on a nearby surface while digesting the blood meal, so if the walls of dwellings have been coated with insecticides, the resting mosquitoes will be killed before they can bite another victim, transferring the malaria parasite.

The first and historically the most popular insecticide used for IRS is DDT. While it was initially used exclusively to combat malaria, its use quickly spread to agriculture.

The World Health Organization (WHO) currently advises the use of 12 different insecticides in IRS operations. These include DDT and a series of alternative insecticides (such as the pyrethroids permethrin and deltamethrin) both combat malaria in areas where mosquitoes are DDT-resistant and to slow the evolution of resistance (WHO, 2006).

One problem with all forms of Indoor Residual Spraying is insecticide resistance via evolution of mosquitoes. According to a study published on Mosquito Behavior and Vector Control, mosquito breeds that are affected by IRS are endophilic species (species which tend to rest and live indoors), and due to the irritation caused by spraying, their evolutionary descendants are trending

towards becoming exophilic (species which tend to rest and live out of doors), meaning that they are not as affected-if affected at all-by the IRS, rendering it somewhat useless as a defense mechanism (Pates and Curtis, 2005).

- **Mosquito nets and bedclothes**

Mosquito nets help keep mosquitoes away from people, and thus greatly reduce the infection and transmission of malaria. The nets are not a perfect barrier, so they are often treated with an insecticide designed to kill the mosquito before it has time to search for a way past the net. Insecticide-treated nets (ITN) are estimated to be twice as effective as untreated nets (Hull, 2006) and offer greater than 70% protection compared with no net (Bachou *et al*, 2006). Although ITN are proven to be very effective against malaria, less than 2% of children in urban areas in Sub-Saharan Africa are protected by ITNs. Since the *Anopheles* mosquitoes feed at night, the preferred method is to hang a large "bed net" above the center of a bed such that it drapes down and covers the bed completely.

Since 2008, the National Malaria Control Programme (NMCP) in Nigeria has adopted a specific plan, the goal of which is to reduce 50 % of the malaria burden by 2013 by achieving at least 80 % coverage of long-lasting impregnated mosquito nets (LLINs), together with other measures, such as 20 % of houses in targeted areas receiving indoor residual spraying (IRS), and treatment with two doses of intermittent preventative therapy (IPT) for 100 % of pregnant women who visit antenatal care clinics (Ye *et al*, 2012, USAID, 2013; Adigun *et al*, 2015). Because of these measures, the percentage of households with at least one LLIN increased to over 70 % by 2010, compared to only 5 % in 2008 (WHO, 2010). The distribution of mosquito nets impregnated with insecticide (often permethrin or deltamethrin) has been shown to be an extremely effective method of malaria prevention, and it is also one of the most cost-effective methods of prevention. Long-lasting insecticidal mosquito nets (LLINs), e.g: Olyset or DawaPlus which release insecticide for approximately 5 years were distributed free of charge to all communities in Anambra State, Nigeria in 2009 and 2014 through the World Bank and Support of Nigeria Malaria Programme (SUNMAP). Nets are also often distributed through vaccine campaigns such as the measles campaign for children. ITNs have been shown to be the most cost-effective prevention method against malaria and are actively part of WHO’s Millennium Development Goals (MDGs).

ITNs have the advantage of protecting people sleeping under the net and simultaneously killing mosquitoes that contact the net. This has the effect of killing the most dangerous mosquitoes. Some protection is also provided to others, including people sleeping in the same room but not under the net. A study among Afghan refugees in Pakistan found that treating top-sheets and chaddars (head coverings) with permethrin has similar effectiveness to using a treated net, but is much cheaper (Rowland *et al*, 1999). A new approach, announced in *Science* on June 10, 2005, uses spores of the fungus *Beauveria bassiana*, sprayed on walls and bed nets, to kill mosquitoes. While some mosquitoes have developed resistance to chemicals, they have not been found to develop a resistance to fungal infections.

Other methods

Education in recognizing the symptoms of malaria has reduced the number of cases in some areas of the developing world by as much as 20%. Recognizing the disease in the early stages can also stop the disease from becoming a killer. Education can also inform people to cover over areas of stagnant, still water e.g. water tanks which are ideal breeding grounds for the parasite and mosquito, thus cutting down the risk of the transmission between people. This is most put in practice in urban areas where there are large centers of population in a confined space and transmission would be most likely in these areas.

2.10 Socio-economic effects of malaria

Malaria is not just a disease commonly associated with poverty but also a cause of poverty and a major hindrance to economic development. The disease has been associated with major negative economic effects on regions where it is wide spread. During the 19th and early 20th centuries, it was a major factor in the slow economic development of the southern states (Humphreys, 2001). A comparison of average per capita Gross Domestic Product (GDP) in 1995, adjusted for parity of purchasing power, between countries with malaria and countries without malaria gives a fivefold difference ($1,526 USD versus $8,268 USD). In countries where malaria is common, average per capita GDP has risen (between 1965 and 1990) only 0.4% per year, compared to 2.4% per year in other countries (Sachs and Malaney, 2002). The economic impact of malaria is estimated to cost Africa $12 billion every year (Gallup and Sachs, 2001). The economic impact

includes costs of health care, working days lost due to sickness, days lost in education, decreased productivity due to brain damage from cerebral malaria, and loss of investment and tourism (Greenwood *et al*, 2005).

Malaria is bad for business: the disease is responsible for employee absenteeism, increased health care spending, and decreased productivity, all of which can negatively impact a company's reputation (Roll Back Malaria, 2011). A 2011 Roll Back Malaria report found that in sub-Saharan Africa, 72% of companies reported a negative malaria impact, with 39% perceiving these impacts to be serious.

In a 2004 survey, nearly three-quarters of companies in the Africa region reported that malaria was negatively affecting their business. Poor children and women in rural areas are at the greatest risk of death or severe debility from malaria, which drains the resources of families (World Bank Regional Office, 2009). Overall, households in Africa lose up to 25% of income to the disease (The Abuja Declaration and the Plan of Action, 2006).

Leading economists estimate that malaria causes an "economic growth penalty" of up to 1.3% per year in malaria endemic African countries. Malaria discourages investments and tourism, affects land use patterns and crop selection resulting in sub-optimal agricultural production, reduces labor productivity, and impairs learning (WHO, 2008). It is estimated that in endemic areas such as Uganda, malaria may impair as much as 60% of the school children's learning ability (Ministry of Health, 2001).

Malaria can strain national economies, impacting some nations' gross domestic product by as much as an estimated 5–6%. In some areas, malaria accounts for 15% of health-related absenteeism from school (Leighton and Foster, 1993). In some countries with a heavy malaria burden, the disease may account for as much as 40% of public health expenditure, 30-50% of inpatient admissions, and up to 50% of outpatient visits.

The impact of malaria extends beyond health facilities to homes and everyday lives: children may develop long-term neurological sequelae following severe malaria attacks (Mishra and Newton, 2009), more subtle developmental and cognitive impairments as a result of both severe and uncomplicated episodes (Holding and Kitsao-Wekulo, 2004), and families face substantial economic consequences (Sicuri *et al*, 2013).

In Nigeria malaria costs the Federal government of Nigeria over one billion naira annually in treatment (Government in action, 2005). Malaria imposes substantial costs to both individuals and governments. Costs to individuals and their families include purchase of drugs for treating malaria at home; expenses for travel to, and treatment at, dispensaries and clinics; lost days of work; absence from school; expenses for preventive measures; expenses for burial in case of deaths. This devastating disease affects the country's economic productivity, resulting in an estimated monetary loss is approximately 132 billion Naira (~700 million USD), in treatment costs, prevention, and other indirect costs (FMOH, 2012; WHO, 2012). Moreover, malaria accounts for 60 % of outpatient visits to hospitals and led to approximately 11 % maternal mortality and 30 % child mortality, especially among children less than 5 years (WHO, 2014; FMOH, 2009).

Costs to governments include maintenance, supply and staffing of health facilities; purchase of drugs and supplies; public health interventions against malaria, such as insecticide spraying or distribution of insecticide-treated bed nets; lost days of work with resulting loss of income and lost opportunities for joint economic ventures and tourism. Direct costs (for example, illness, treatment, premature death) have been estimated to be at least US$ 12 billion per year.

The extensive use of anti-malaria campaigns in recent decades seeks to address the correlation between the disease and poverty. Government subsidies and public healthcare providers made available in closer proximity to all of the people in a community are efficient methods to reduce the cost of treatment for the poor and the rest of the social classes as that would allow equal accessibility and utilization of treatment (WHO, 2006).

2.11 Malaria Vaccines

Vaccines are often the most cost-effective tools for public health. They have historically contributed to a reduction in the spread and burden of infectious diseases and have played the major part in previous elimination campaigns for smallpox and the ongoing polio and measles initiatives. Yet no effective vaccine for malaria has so far been developed. Malaria vaccines are an area of intensive research; however, there is no effective vaccine that has been introduced into clinical practice. There is one candidate vaccine, known as RTS,S/AS01, which started Pivotal Phase III evaluation in May 2009 and is designed not for travelers but for children resident in malaria-endemic areas who suffer the burden of disease and death related to malaria.

Vaccines against malaria

RTS, S/AS01 (RTS, S) – also known as Mosquirix – is an injectable vaccine that provides partial protection against malaria in young children. The vaccine is being evaluated in sub-Saharan Africa as a complementary malaria control tool that potentially could be added to (and not replace) the core package of WHO-recommended preventive, diagnostic and treatment measures.

In July 2015, the vaccine received a positive opinion by the European Medicines Agency, a stringent medicines regulatory authority. In October 2015, two WHO advisory groups recommended pilot implementation of RTS, S/AS01 in a limited number of African countries. WHO adopted these recommendations and is strongly supportive of the need to proceed with the pilot programme as the next step for the world's first malaria vaccine.

In November 2016, WHO announced that the RTS, S vaccine would be rolled out in pilot projects in 3 countries in sub-Saharan Africa. Funding is now secured for the initial phase of the programme and vaccinations are due to begin in 2018. These pilot projects could pave the way for wider deployment of the vaccine if safety and effectiveness are considered acceptable.

2.12 Growth and Nutritional Status in Children

Malnutrition is estimated to contribute to more than one third of all child deaths, although it is rarely listed as the direct cause. Lack of access to highly nutritious foods, especially in the present context of rising food prices, is a common cause of malnutrition. Poor feeding practices, such as inadequate breastfeeding, offering the wrong foods and not ensuring that the child gets enough nutritious food, contribute to malnutrition. Infection particularly frequent or persistent diarrhoea, pneumonia, measles and malaria also undermines a child's nutritional status. Each year, no less than one million Nigerian children die before their fifth birthday and malnutrition contributes to nearly half of these deaths, thus imposing a staggering cost to the nation (Ogundipe, 2015). Health experts at a media dialogue on child malnutrition with the theme: "Spend More Money on Nutrition", organized by UNICEF in collaboration with its partners, observed that the first 1,000 days in the life of a child represents a critical window of opportunity and adequate nutrition during this period can avert malnutrition by ensuring that children have the best possible opportunity to grow, learn and rise out of poverty. The World Health

Organization (WHO) defines malnutrition as the cellular imbalance between the supply of nutrients and energy and the body's demand for them to ensure growth, maintenance and specific functions. Malnutrition generally implies undernutrition and refers to all deviations from adequate and optimal nutritional status in infants, children and in adults. In children under nutrition manifests as underweight and stunting.

The best method of assessing the health and nutrition of the individual child is by longitudinal monitoring of growth. Malnutrition refers to deficiencies, excesses or imbalances in a person's intake of energy and/or nutrients. The term malnutrition covers 2 broad groups of conditions. One is 'undernutrition' which includes stunting (low height for age), wasting (low weight for height), underweight (low weight for age) and micronutrient deficiencies or insufficiencies (a lack of important vitamins and minerals). Stunting (height-for-age <-2 z-scores) or wasting (weight-for-height <-2 z-scores) of the WHO/NCHS reference (WHO, 1995) is used to survey children in a community to make an objective assessment of their nutritional status. Key indicators for monitoring the nutritional status of a child less than 5 years of age are underweight, stunting and wasting.

- **Weight for age and height for age**

It is simple to measure the weight and height of a child and compare it with an accepted standard if the age is known. Children weighing less than 80% of Harvard mean were considered malnourished while those less than 60% of this mean were severely malnourished. Eighty percent of the mean for weight is approximately the 3rd centile, or -2 SD. Using the height for age, children less than 90% of the Harvard mean were considered 'stunted' and children below 80% of the mean severely stunted; 90% of the mean for height is approximately the 3rd centile or -2 SD, while 80% is approximately -3 sd (Waterlow *et al,* 1977).

- **Mid-upper arm circumference (MUAC)**

The mid upper arm circumference changes little between the ages of one and five years. At one year, there is a good deal of fat under the skin, while at five years, there is more muscle and less fat, but the total circumference remains almost the same. The major determinants of MUAC are muscle and subcutaneous fat, both important determinants of survival in malnutrition and starvation. MUAC is less affected than weight and height based indices (e.g. Body Mass Index)

by accumulation of fluid (i.e. nutritional oedema, peri-orbital oedema and ascites). So MUAC is a good predictor of mortality. It is recommended for identifying young children with or at risk of severe acute malnutrition and adults with acute energy deficiency (Morley and Woodland, 1979). In children 6-59 month old, MUAC over 135mm (13.5cm), green colour indicates that the child is well nourished. MUAC of between 125mm (12.5cm) and 135mm (13.5cm), yellow colour, indicates that the child is at risk for acute malnutrition and should be counseled and followed-up for Growth Promotion and Monitoring (GPM). MUAC of between 110mm (11.0cm) and 125mm (12.5cm), red colour (3-colour Tape) or orange colour (4-colour Tape), indicates Moderate Acute Malnutrition (MAM). The child should be immediately referred for supplementation. MUAC less than 110mm (11.0cm), red colour, indicates Severe Acute Malnutrition (SAM). The child should be immediately referred for treatment.

- **Weight for Height**

Another method of assessing the nutritional status of children of uncertain age is to compare their weight with their height called the height and growth charts percentile. A growth chart has seven curves that follow the same pattern; each one represents a different percentile: 5th, 10th, 25th, 50th, 75th, 90th and 95th as shown in Figures 1 and 2.

About Pediatrics

2 to 20 years: Girls
Stature-for-age and Weight-for-age percentiles

NAME

*To Calculate BMI: Weight (kg) ÷ Stature (cm) ÷ Stature (cm) x 10,000
or Weight (lb) ÷ Stature (in) ÷ Stature (in) x 703

SOURCE : Developed by the National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion (2000). http://www.cdc.gov/growthcharts

Fig 1: Stature for age and weight-for –age percentiles for girls (2-20 years).

Source: Developed by the National Center for Health Statiatics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion (2001).

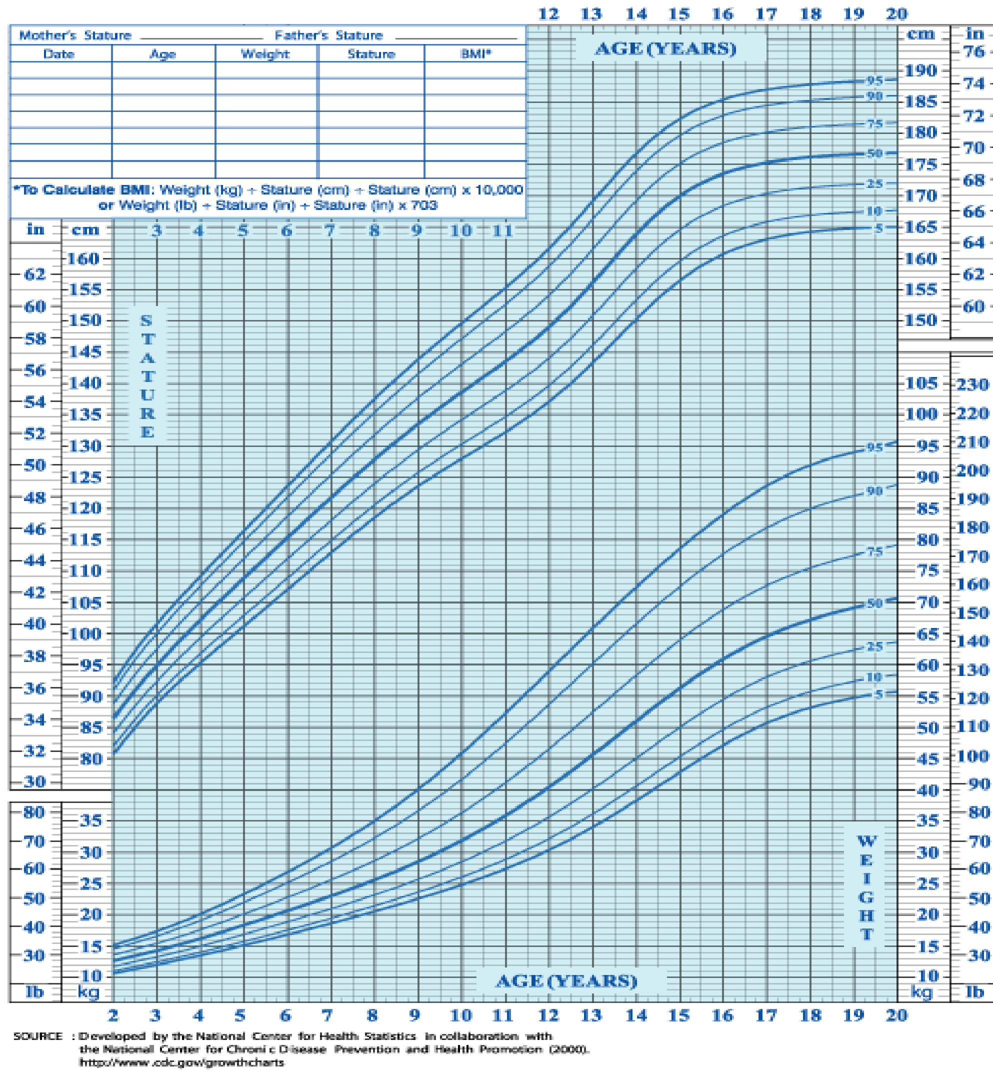

Fig 2: Stature for age and weight-for –age percentiles for boys (2-20 years).

Source: Developed by the National Center for Health Statiatics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion (2001).

KEY:

- Body mass Index (BMI) 1st to 4th percentile: Underweight*
- BMI 5th to 84th percentile: Healthy Weight*
- BMI 85th to 94th percentile: Overweight*
- BMI 95th to 100th percentile: Obese*

The 50th percentile line represents the average value for age. An infant whose head circumference falls in the 90th percentile will be plotted right on the second curve from the top of the chart (the 90th percentile curve). Being in the 90th percentile means the child's head measurement is greater than or equal to the measurements of 90% of children of that age in the country. The remaining 10% of infants that age have head measurements that exceed that child's. If a 4-year-old's weight at a checkup falls in the 20th percentile, that reading will be plotted between the curves for the 10th and 25th percentiles. That means 80% of children of that age weigh more and 20% less than that child. The child whose weight falls in the 20th percentile may have parents who are a bit below average for height and weight. For him, being in the 20th percentile is an entirely normal reading. Sometimes, however, a child's measurement increases or falls sharply, or is at one extreme of the growth chart. For example, children who fall below the 5th percentile on the weight for stature (height) chart are considered underweight; children at or above the 85th percentile on this chart are considered overweight (and at risk for obesity); and those at or above the 95th percentile are considered to be obese.

Generally, if a measurement exceeds the 95th percentile or crosses two percentile curves (such as climbing from the 40th percentile to the 75th percentile, thereby crossing the 50th and 75th percentile curves), there may be some cause for concern. On the other hand, if a measurement falls below the 5th percentile or crosses two percentile curves (dropping from the 50th to the 20th percentile; for instance), the doctor will also consider the possibility of a health problem affecting the child's growth. When growth chart readings are examined over time, they reveal a pattern of development. Those patterns will help to determine how the child is growing in relation to other children of his age and also shows how he has progressed from previous measurements.

Uwem *et al,* (2012) noted a prevalence of 38.7%, 13.6%, and 38.7%, of stunting, wasting and underweight respectively among children 6 months to 15 years in settled pastoral Fulani children in Southwest Nigeria with boys being more malnourished than the girls. In another study, Manyike *et al* (2014) assessed the prevalence of malnutrition among pre-school children aged 1-5 years in Abakiliki Ebonyi State of Nigeria, a prevalence of global and severe acute malnutrition of 9.7% and 4.4% respectively and stunting of 9.9% was reported with a male prepondence. Kateera *et al*, (2015) noted a 41.3 % prevalence of stunting among preschool children in rural Rwanda. Nyakeriga *et al*, (2004) in a study on malaria and nutritional status in

children living on the coast of Kenya observed 1.17% and 0.94% low height-for-age and low weight-for-age respectively.

2.13 Nutritional Potentials of *Moringa oleifera*

Moringa oleifera is an outstanding indigenous source of highly digestible proteins, calcium (Ca), iron (Fe), vitamin C and carotenoids suitable for utilization in regions of the world where under nutrition is a major concern. *Moringa oleifera* leaf powder is used in many countries of the world as a micronutrient powder to treat diseases. *Moringa oleifera*, commonly referred to as *"Moringa"*, is the most widely cultivated species of the genus *Moringa*. It is the only genus in the family *Moringaceae* and a nutritious vegetable tree with a variety of potential uses. *Moringa* is a fast-growing, drought-resistant tree native to Northern India but now widely distributed in the tropics and sub-tropical zones. *Moringa* leaf powder is used to aid the restoration of infants suffering from malnutrition. All parts of *Moringa* are consumed as food. According to Trees for Life (2005), extensive research conducted at the Noguchi Memorial Medical Research Centre in Ghana has found that its leaf powder has no toxic elements and no side effects were observed even in families consuming high amounts of *Moringa*. Dr. Lowell Fuglie, based in Senegal and considered as one of the experts about *Moringa* says that to date there has not been any known negative side effect even in the daily consumption of *Moringa* (Fuglie, 2001; Satya *et al*, 2012). The plant produces leaves during the dry season and during times of drought, and is an excellent source of green vegetable when little other food is available (FAO, 2014).

Moringa is known by various names in English, French and local African, Asian and South American languages. **English**: Horseradish tree, Radish tree, Benoil tree, Drumstick, Miracle tree, mother's best friend, West Indian ben, Never die and Moringa nut. **French:** Ben aile and Benzolve, **Nigeria:** (**Fulani)** *Gawara, Konamarade, Rinimaka, Habiwal hausa.* **(Hausa)** *Zogall, Zogallagandi, Bagaruwar maka, Bagaruwar masar, Shipka hali Shuka halinka Barambo* etc. **(Igbo):** *Odudu oyibo, Okwe oyibo, Okwe olu, Okochi egbu, Okughara ite, Uhe, Ikwe beke*. (**Yoruba):** *Ewe ile, Ewe igbale, Adagba malonye* (Ozumba, 2008).

In Nigeria, it is encountered doing well in all ecological zones where it is in season all year round (Ozumba, 2008). It is considered one of the world's most useful trees. Every part of the

Moringa oleifera tree from the roots to the leaves has beneficial properties that can serve humanity. All parts of the *Moringa* tree are edible and have long been consumed by humans. *Moringa* trees have been used to combat malnutrition, especially among infants and nursing mothers. Leaves can be eaten fresh, cooked or stored as dried powder for many months without refrigeration and reportedly without loss of nutritional value. *Moringa* is especially promising as a food source in the tropics because the tree is in full leaf at the end of the dry season when other foods are typically scarce. As a source of nutrition, *Moringa* leaves probably rank as the best of all tropical vegetables. They contain very strong concentrations of Vitamin A, C, B complex, Iron, calcium, protein, Zinc, Selenium and unusual for a plant source, all the essential amino acids (Fuglie, 2001). They are highly recommended for pregnant and nursing mothers as well as young children (FAO, 2014). In the Philippines, due to their high iron content, *Moringa* leaves are used in the treatment of anaemia.

Igwilo (2012) noted that "*Moringa* is gold and will fetch Nigeria more revenue than oil because oil has its own negative impact on the environment but with *Moringa*, there is no such thing." The plant is medicinal, it contains most of the nutrients needed by the body and it is an immune booster, the leaves and seeds also cure malaria and the phytochemical analysis of the plant show that the leaf contains saponins and tannins which reduce the incidence of cancer by masking and removing carcinogenic agents so that they will not affect the cells and cause cancer (Igwilo, 2012).

A gram –for- gram comparison of *Moringa* leaf nutrients with those of other food items according to Gopalan *et al* (1989) is as follows:

***Moringa* has seven times the vitamin C found in oranges**: Vitamin C is required by our body to strengthen our immune system and fights infectious diseases including colds and flu. Citrus fruits such as oranges and lemons are full of vitamin C. *Moringa* leaves have even more.

***Moringa* has four times the calcium found in milk:** To build up strong bones and teeth calcium is very important, this helps to prevent osteoporosis. Milk provides a lot of calcium, but *Moringa* leaves provide even more.

***Moringa* contains four times the Vitamin A found in carrots:** Vitamin A acts as a shield against diseases of the eyes, skin and heart, diarrhea and many other ailments. Carrots are very high in vitamin A; with *Moringa* leaves it is even higher.

***Moringa* has three times the potassium found in bananas:** Potassium is essential for the brain and nerves. Bananas are an excellent source of potassium. *Moringa* leaves are even better.

***Moringa* contains two times the protein found in yogurt:** Proteins, the building blocks of our bodies, are made of amino acids. Usually only animal products such as meat, eggs and dairy contain all the essential amino acids. Amazingly, *Moringa* leaves also contain them all.

***Moringa* has 75% the iron found in spinach:** Iron plays an important role in the synthesis of hemoglobin in blood which carries oxygen to all parts of the body. Spinach is well known for its iron content. *Moringa* leaves also contain iron.

Daily consumption of *Moringa* leaves yields the following benefits:

- Promotes Body's Natural Defenses
- Provides Antioxidants
- Nourishes Body's Immune System
- Act as an Anti-Inflammatory agent
- Supports Normal Blood Glucose Levels
- Triggers Metabolism
- Promotes Healthy Digestion
- Enhances Skin Health
- Reduces the Appearance of Wrinkles
- Promotes Normal Liver Function
- Promotes Healthy Cell Structure
- Promotes Healthy Circulatory System
- Promotes Normal Serum Cholesterol
- Nourishes the Eyes and Brain
- Increases Energy and
- Provides a Sense of Well-Being

Young children that consume dried *Moringa oleifera* leaf powder on a regular basis increase their weight and overall health and adding one large spoonful (8g) of dried *Moringa* leaf powder to complementary foods three times each day will ensure that a child is getting a nutrition rich diet (USAID, 2012). These numbers are particularly astounding; considering that this nutrition is

available when other food sources may be scarce. Since the leaves contains nutrients that promote body defenses and nourish the immune system, daily consumption by school children will boost their immunity and fight infections (e.g. malaria parasite) through the natural defense.

Health benefits of phytochemicals in *Moringa oleifera* leaf

Nutritional analysis indicates that *Moringa* leaves contain a wealth of essential disease preventing nutrients which make it suitable to be included in diets as food supplement (Krishnaiah *et al*, 2009). The presence of phytochemicals such as saponin, phenol, tannin, terpenoids, flavonoids, alkaloids and phytic acid in *Moringa oleifera* leaf indicates its possible preventive and curative properties (Josephine *et al*, 2010).

Saponins bind with cholesterol and other pathogens in the body and prevent them from being absorbed by the body, carrying them through the body's digestive system to be eliminated. Saponins get rid of destructive pathogens in the body thus relieving stress on the immune system for effective functioning, reduce the risk of getting cancer, relieve eczema and skin allergies, reduce bone loss, lower the risk of getting various heart diseases and also acts as an antioxidant.

Phenol and tannin have antioxidant effects; they react with and capture dangerously reactive compounds called free radicals preventing the radicals from reacting with other biomolecules and causing serious damage in the body.

Terpenoids are of great interest due to the broad range of biological activities reported such as cancer preventive effects, analgesic, anti-inflammatory, antimicrobial, antifungal, antiviral, and antiparasitic activities (Singh and Sharma, 2015).

Flavonoids are hydroxylated phenolic compounds that are present in plants and occupy a special place among secondary metabolites. They are classified into different classes, with flavones, flavonols, flavanones, catechins, isoflavones, and anthocyanidins being the most common. Similar to terpenoids, they also present a wide range of biological activities. These compounds have been demonstrated to have protective effects against many infectious and degenerative diseases such as cancer, among other important pharmacological activities such as antioxidant and anti-inflammatory activities (Mamadalieva *et al*, 2011).

Alkaloids are produced by a large variety of organisms including bacteria, fungi, plants, and animals. Alkaloids have a wide range of pharmacological activities including antimalarial (*e.g.*

quinine), antiasthma (*e.g.* ephedrine), anticancer (*e.g.* homoharringtonine) (Kittakoop *et al*, 2014)

Phytic acid has protected against alcohol-related liver injury by blocking free radicals and elevating antioxidant potentials. The anti-oxidative action of phytic acid is as a result of inhibiting Xanthine Oxidase and by 'preventing a formation of ADP-iron-oxygen complexes. It was also able to protect DNA from free radicals. Phytic acid supplementation increases gut transit time and result in more efficient absorption of nutrients (Muraoka and Miura, 2004).

Moringa oleifera leaf is also known to be the best nutritional support for nursing mothers because it is not only rich in nutritional content but for its medicinal properties as well. By making vegetable soup out from fresh *Moringa* leaves, it has been found to increase the volume of breast milk produced by lactating mothers. For pregnant and breast-feeding women, *Moringa* leaves and pods can do much to preserve the mother's health and pass on strength to the fetus or nursing child because the right nutrition in the first one thousand days has a profound impact on a child ability to develop its potential. One 100 g portion of leaves could provide a woman with over a third of her daily need of calcium and give her important quantities of iron, protein, copper, sulfur and B-vitamins. It is estimated that only 20-40% of vitamin A will be retained if leaves are dried under direct sunlight, but that 50-70% will be retained if leaves are dried in the shade. A daily dose of one rounded tablespoon (8 g) of *Moringa oleifera* leaf powder will satisfy about 14% of the protein, 40% of the calcium, 23% of the iron, 13% of the potassium, 7% of the Vitamin C and 100% of the vitamin A for a child under five years (Fuglie, 2001).

CHAPTER THREE
MATERIALS AND METHODS

3.1 General Methodology

3.1.1 Study Area

The study was carried out in Oba community. Oba is one of the towns that make up the Idemili-South Local Gvernment Area of Anambra State in SouthEast Nigeria and lies approximately 7 kilometers south of Onitsha along the old Owerri-Onitsha Trunk A Road (fig 3.1.3). Oba community has an area coverage of about 68 square kilometers lying between latitude 6° $03^{/}$N and longitude $6^{o}52^{/}$E (Google maps world gazetteer, 2017). Oba is bounded to the north by the Idemili River and the neighboring towns of Nkpor and Umuoji, to the south are the Ekulu River, Oraifite and Akwa-Ukwu communities, to the east are Ojoto and Ichi communities and to the west are Ose River, Obosi and Odekpe communities (Figs 3 and 4).

Oba is a sub-urban community made up of nine villages viz: Urueze, Umuogali, Isu, Okuzu, Abime, Ogwugwu, Ogbenwe, Aboji, and Ezelle (Fig 5). Oba community, like other communities in the tropical rain forest zone of Nigeria, has two marked seasons per year viz: wet and dry seasons. The rainy season stretches from April to October and the dry season from November to March. The annual rainfall averages about 1,850mm (74 inches) per annum. The temperature is generally high with maximum monthly temperature varying between 27.2^{o}C and 35°C and highest between February and March (Google maps world gazetteer, 2017). The minimum temperatures vary between 18.2^{o}C and 23°C, the coolest between August and September. The relative humidity of Oba community varies between 40 and 92%. The humidity is generally high during the early hours of the day being highest in July during the rainy season and lowest in January during the harmattan.

Oba community has undulating landscape with hilly areas alternating with valleys. In the western half, the landscape rises between 150 and 450 meters, punctuated by hills and valleys that determine the natural course of the river Idemili running from north to northwest. Occurrence of loamy and humus soils in the valleys have enhanced the cultivation of food crops and vegetables.

Oba community has typical rain forest vegetation characterized by thick forests in virgin uncultivated areas and derived guinea savanna in regularly cultivated areas and other land use practices. The most common trees are palm and mahogany whose long taproot and hard back enable it to survive long dry season and to resist serious damage by the bush fires.

The estimated population of Oba community is 52,345 (National Population Commission, 2006). Oba community has six private hospitals, one Government hospital, three Health Centres, many Patent medicine stores and six private maternity homes. There are thirty nursery schools (thirteen public and seventeen private) in the nine villages of Oba community. The indigenes are Ibos who speaks Igbo as their local language. The primary occupation of the indigenes is farming and wine tapping and their agricultural products are corn, vegetables, yam and cocoyam. Majority of the indigenes are Christians although some are traditionalists. The area is covered with a network of streams and other forms of water bodies with extensive fresh water swamps that encourage the breeding of mosquitoes and other vectors.

Fig. 3 Map of Anambra State showing Idemili-South Local Government Area

Adapted from Google maps world gazetteer (2017).

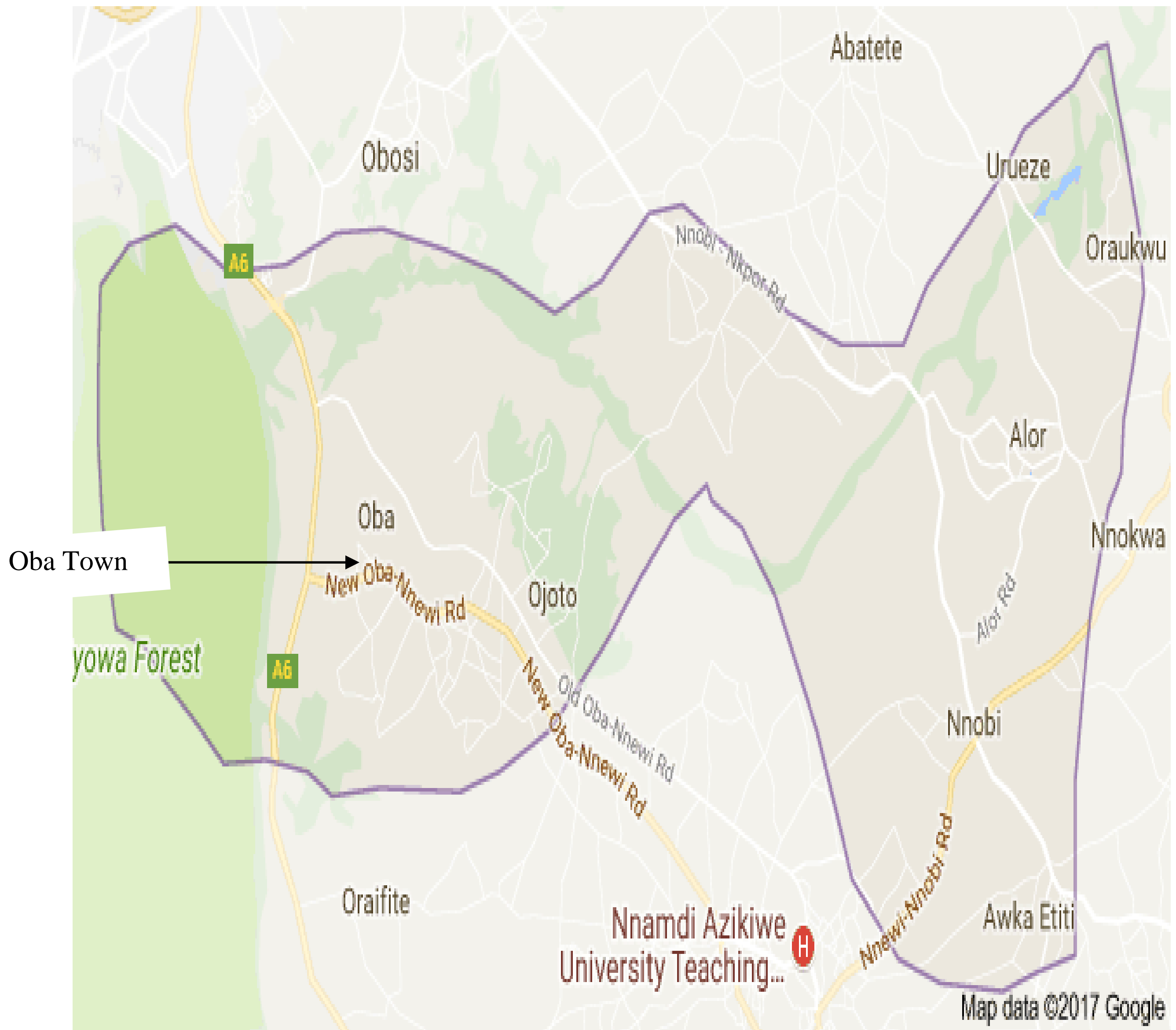

Fig. 4Map of Idemili South Local Government Area showing Oba the study Area

Adapted from Google maps world gazetteer (2017).

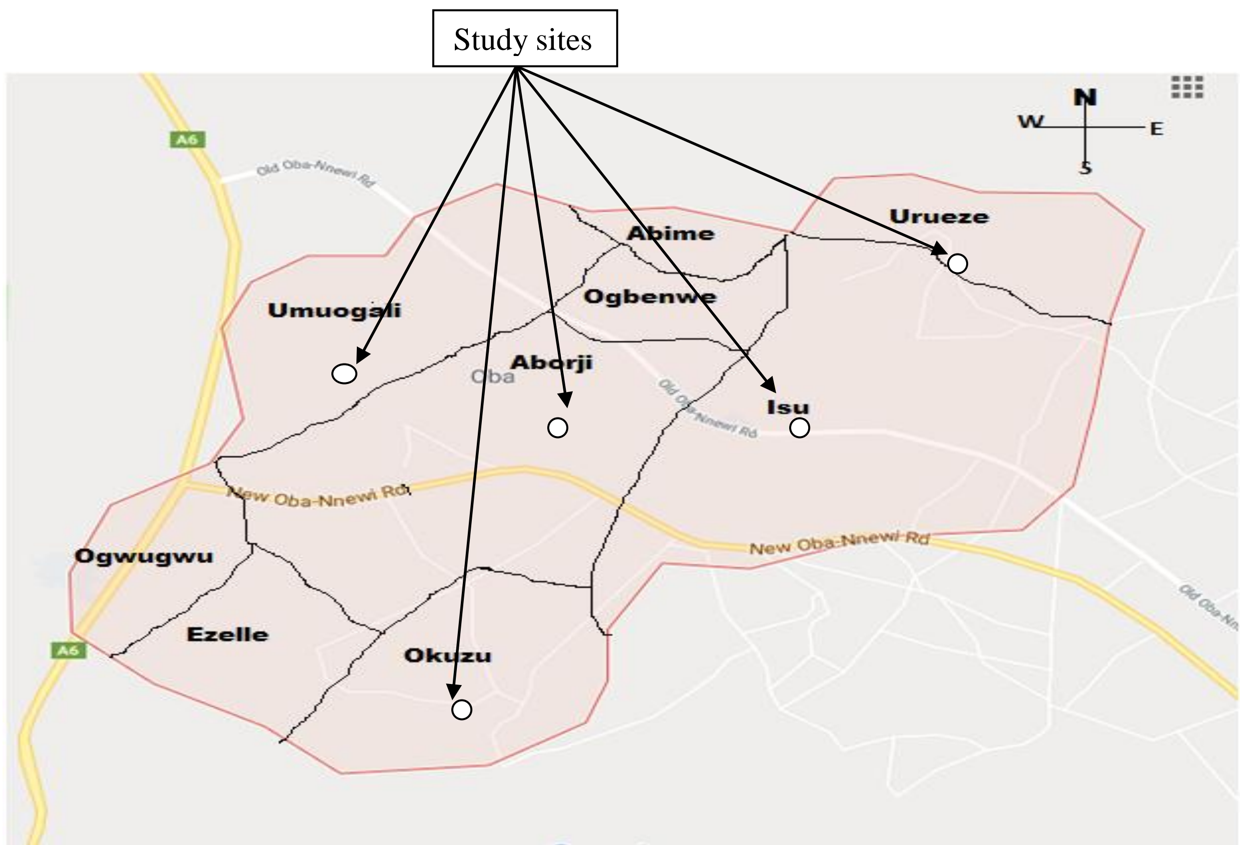

Fig. 5 Map of Oba showing the nine villages and study sites

Adapted from Google maps world gazetteer (2017).

3.1.2 Study Design

The study was a facility-based longitudinal study aimed at evaluating the effects of *Moringa oleifera* leaf powder on the nutritional status and malaria reinfection among children under five years. Five hundred (500) children under five years were randomly selected by balloting from 10 out of 30 nursery schools in Oba Community.

The pupils were subdivided into two experimental groups A and B. Group A was fed with jollof rice and 8g of *Moringa oleifera* leaf powder and Group B was fed with jollof rice only. Malaria prevalence and reinfection, haematological parameters and nutritional status of the children were experimentally determined every two months for six months.

3.1.3 Ethical Approval

Ethical approval for the use of human subjects in the study was obtained from the Scientific and Ethical review committee of Nnamdi Azikiwe University Teaching Hospital Nnewi, Anambra State (Appendix 1).

3.1.4 Advocacy visits and Community Sensitization

Advocacy visits with a letter of introduction from the Head of Department of Parasitology and Entomology, Nnamdi Azikiwe University Awka, was used to obtain permission from the Education Secretary Idemili South Local Government Area (Appendices 2 and 3).

Advocacy visits were also conducted to the community leaders, village chiefs, head teachers and staff of the selected schools for their consent to study in the community and schools. Meetings were held with the Parents Teachers Association (PTA) members in each selected schools to inform them about the study and its benefits. Written consent was received from the parents and guardians (Appendix 4), before involving their children in the study. Gifts of biscuits and candies were offered to the children after blood collection. The teachers in each of the schools visited helped in controlling the children.

3.1.5 Sample Size and Sampling Techniques

Sample size was calculated using the formula below based on previous malaria prevalence among children less than five years (Pourhoseinghi *et al*, 2013).

$$n = \frac{t^2 \times p(1-p)}{m^2}$$

Where: **n** = required sample size

t = confidence level at 95% (standard value of 1.96)
p = estimated prevalence of malaria in the project area 30.7% south east Nigeria (FMOH, 2001)
m = margin of error at 5% (standard value of 0.05).

Substitution in the above formula gave a minimum sample size of 330 pupils. The figure was increased to 500 substituting with 3% of the total population of children less than 5 years in the study area as follows: 3% of 15720 (total population of children below 5 years in the study area) 500 pupils were selected for the study.

The sampling technique adopted for the selection of the target population was multi-stage random sampling. In the first stage, five out of the nine villages in Oba were selected judgementaly. The selected villages were Isu, Okuzu, Aborji, Umuogali and Urueze. Out of the thirty (thirteen public and seventeen private) nursery schools in the nine villages of Oba, there were a total of twenty (ten private and ten public) nursery schools in the five selected villages. In the second stage, five private nursery schools (Liberation Nursery School, One Foundation Nursery School, Devans Nursery School, St Pauls Anglican Church Nursery School, St Joseph Catholic Church Nursery School) and five public nursery schools (Isu Ebii Nursery School, Umuogali Nursery School, United Nursery School, Ugwube Nursery School and Bright Nursery School) were selected from the five selected villages by balloting. In the final stage, 50 pupils in nurseries two and three from each of the ten selected nursery schools were randomly selected by balloting, that is 25 pupils each from nurseries two and three totaling 500 pupils.

3.1.6 Demographic data collection

Oral interviews was conducted among the selected children to determine their names, age, class, type of food they eat at home and where they live. School registers was used to confirm the demographic data and recorded in a notebook.

3.1.7 Inclusion criteria

Pupils whose names were on the school register and children whose parents/guardians consented and signed the informed consent form.

Exclusion criteria: Pupils whose parents/guardians did not consent. Pupils who were fed with *Moringa oleifera* leaf powder previously at home.

3.1.8 Preparation of Jollof Rice (JR)

Recipe: A quarter (1/4) cup of local rice, 1/2 small size onion, 1 large fresh tomato, 1/4 tinned tomato puree, ½ kitchen spoon red oil (increases the bioavailability of *Moringa*) (Vanisha, 2006), one teaspoon ground crayfish, salt to taste. Recommended portion size for children 4 to 5 years is 50g which is equivalent to 2 kitchen spoonfuls (Wrieden *et al*, 2008). Therefore each participant was served with 2 kitchen spoonfuls of jollof rice daily for six months.

Nutrient content of jollof rice in Kcal

The nutrient content of the jollof rice served is as follows: Energy (g) = 319.4, Protein (g) = 2.15, Fat (g) = 46.6, Carbohydrate (g) = 49.4, Calcium (mg) = 18.65, Iron (mg) = 2.065, Vitamin A (I.U) = 2611, Ascorbic acid (mg) = 25.9.

3.1.9 Formulation of *Moringa oleifera* leaf powder

Product: *Moringa oleifera* leaf powder was purchased from NAFDAC approved *Moringa* processing factory (VIDSA multiventures LTD Ibadan, NAFDAC No 08-0866L). Produced and packaged by VMV Limited. Plot 13 Oluwaseun/Bolajoko Estate Akobo Ibadan Oyo State, Nigeria. According to the manufacturer, each packet (40g) of *Moringa oleifera* leaf powder has the following Nutritional Facts : Saponin, Crude fibre 8.4%, Crude protein 20.1%, Iron 7.0mg/100mg, Magnesium 18.3mg/100mg, Phosphorus 12.0mg/100mg, Calcium 51.0mg/100mg, Vitamin C 25.09mg/100mg, Volatile oil 1.4%, Zinc 4.2mg/100mg. **Phytochemical analysis of the *Moringa oleifera* leaf powder resulted in the following:**

Phytochemicals	Compositions
Phenol (mgGAE/g)	42.42±0.54
Flavonoid (mgCE/g	0.55± 0.03
Tannin (mgTAE/g)	123.91±0.06
Terpenoids (mg/g)	0.995±0.23
Alkaloid (%)	0.42±0.25
Phytate (%)	4.46±0.29
Oxalate (mg/g)	1.89±0.38
Saponin (%)	0.49±0.10

According to Satya *et al* (2012) one rounded tablespoon (8 g) of *Moringa oleifera* leaf powder will satisfy about 14% of the protein, 40% of the calcium, 23% of the iron and nearly all the vitamin A needs for a child under five years. Young children that consume dried *Moringa oleifera* leaf powder on a regular basis increase their weight and overall health and adding one large spoonful (8g) of dried *Moringa* leaf powder to complementary foods each day will ensure that a child is getting a nutrition rich diet (USAID, 2012). Recommended quantity of *Moringa oleifera leaf powder* served to each child was 8g which is equivalent to one tablespoon.

3.1.10 Evaluation of acceptability of experimental diet

A pilot study using 20 randomly selected nursery school children aged 4-5 years (2 children from each selected school) was conducted for 4 weeks prior to the main study. The reason for the pilot survey was to evaluate the acceptability of the jollof rice diet with or without *Moringa oleifera* leaf powder among the pupils and to identify the challenges that may be encountered and proffer solutions to them before the main study. No case of diarrhoea, vomiting, allergic reaction and abdominal pain was reported during the trial period.

3.1.11 Feeding arrangement and Feeding of the children

Each pupil in Group A (250 children) received two kitchen spoonfuls of jollof rice mixed with 8g of *Moringa oleifera* leaf powder (MOLP) while each pupil in Group B (250 children) received two kitchen spoonfuls of jollof rice without *Moringa oleifera* leaf powder as daily lunch pack. The feeding was carried out daily for six months (June to November) into the school year from Monday to Friday. Holiday lessons was organized during vacation to avoid break in the feeding regimen and pattern with the help of the PTA coupled with the fact that the children were assured of a decent meal once they come.

3.2 Objective 1: Determination of malaria prevalence and intensity

3.2.1 Collection of blood samples

At the beginning of the study, 6ml venous blood was collected from each pupil using a tubing tourniquet tied to the upper arm after cleaning with cotton wool soaked with methylated spirit. Four milliliters of the collected blood was emptied into a plain tube and used for determination of protein and iron levels, the remaining two milliliters of the collected blood was emptied into an Ethylene Diamine Tetra-acetic Acid (anticoagulant) specimen bottle already labeled with each child's name and mixed gently. The anticoagulant, Ethylene Diamine Tetra-acetic Acid (EDTA) in the sample tube contains chemicals that prevented blood from clotting by removing calcium (Cheesbrough 2000). The blood was used for preparation of thick and thin blood smears for determination of malaria parasite, packed cell volume, haemoglbin, white blood cell count and platelet estimation. This was repeated every two months for six months following feeding the children with jollof rice with or without *Moringa oleifera* leaf powder.

3.2.2 Preparation of thick blood films

A drop of blood from each subject was placed at the centre of a well labeled, clean grease free microscopic slide. The corner of a clean slide was used to spread the drop of blood in a circle to the size of a dime (diameter 1-2 cm). The thick film was air dried for 30 minutes before staining. The thick film was not fixed in methanol, this allows the red blood cells to be haemolyzed so that only leukocytes and any malaria parasites present will be the only detectable elements. However, the haemolysis may lead to distorted plasmodial morphology making *Plasmodium* species differentiation difficult. Therefore, the thick smear was mainly used to detect infection and to estimate parasitemia.

3.2.3 Preparation of thin blood films

A drop of blood from each subject was placed on one end of a well labeled, clean grease free microscopic slide. A clean spreader slide was held at a 45° angle, toward the drop of blood on the specimen slide and the blood was allowed to spread along the entire width of the spreader slide. While holding the spreader slide at the same angle, the spreader was pushed forward

rapidly and smoothly (Cheesbrough 2000). The thin smear was air dried for 10 minutes and fixed in 100% absolute methanol by dipping into methanol for 5 seconds so that the red blood cells will not be haemolysed, it was allowed to dry completely before staining. The fixative is essential for good staining and presentation of cellular detail and species identification.

3.2.4 Staining of the slides

The laboratory method employed for staining and identification of malaria parasites in collected blood samples was as described by Cheesbrough (2000).

Thick films: The thick films were first rinsed in water to haemolyse the red cells and then stained with Giemsa 10% in buffered water at Ph 7.1 for 5 minutes and rinsed gently for 1 to 2 seconds in a jar of tap water. The slide was drained and dried.

Thin film: The thin film was covered in 10% Giemsa stain and left for 30 minutes. The film was washed with distilled water, drained and dried.

3.2.5 Examination and determination of malaria prevalence

The thick film was examined using oil immersion objective lens. The immersion oil was spread to cover about 10mm in diameter in the areas of the film. The microscope was moved by one high-power field each time using the fine adjustment to focus. A minimum of 100 high-power fields was examined before declaring a thick film as having "no malaria parasites". When parasites were observed, a further 100 fields was examined before final identification of the species, ensuring that a mixed infection is not overlooked.

To confirm the parasite species or mixed infections after examining the thick film, the thin film was examined. The thin film was examined microscopically under x100 oil immersion objective lens. The immersion oil was spread to cover about 10mm in diameter in the areas of the film. The feathery end of the thin film where the red cells lay was examined side by side along the edge of the film and then the slide was moved outwards by one field, inwards by one field, returning in a lateral movement. The thin film was examined until the presence and the species of malaria parasites have been confirmed. The species was identified and the species and stages observed were recorded.

3.2.6 Calculation of Prevalence

Prevalence was determined by calculation of the proportion of infected to the number of pupils examined and multiplying by a hundred as follows:

$$P = \frac{\text{Number positive with malaria parasites}}{\text{Total number examined}} \times 100$$

3.2.7 Determination of malaria parasite intensity

Parasite density estimation on thick blood films was as recommended by the World Health Organization (WHO, 2006). A drop of blood (50µl) was spread over a standard area on the slide. Taking into account the diameter of the microscopic field, the average number of fields per microlitre of blood was calculated by comparing with the known white cell count in a calibrator blood specimen and the formula below applied:

$$\frac{\text{Number of parasites} \times 8000}{\text{Number of wbc}} = \text{parasites}/\mu l$$

Thus the grading of intensity of malaria parasitaemia was as follows: + (below 1,000 parasites per c.m.m), ++ (1,000-10,000 parasites per c.m.m), +++ (10,000 -100,000 parasites per c.m.m) and ++++ (over 100,000 parasites per c.m.m).

3.3 Objective 2: Determination of frequency of malaria reinfection

3.3.1 Clearance of malaria parasite infection

Children infected with malaria parasites at the beginning of the study were treated with artemether/lumefantrine (brand name: coartem) and checked for malaria parasite clearance after 3 weeks by subjecting them to malaria parasite test using thick and thin blood films stained with 10% giemsa and examined under x100 oil immersion objective lens.

3.3.2 Determination of malaria parasite reinfection

Infected and treated children were shared into two groups of 36 and 35 while 429 uninfected children were also shared into two groups of 214 and 215 respectively. The 36 infected and treated children were added to the 214 uninfected children while the 35 infected and treated children were added to the 215 uninfected children giving two study groups of 250 children each. One group comprising of 250 children were served jollof rice with 8g of *Moringa oleifera* leaf powder added while the remaining group of 250 children were served jollof rice only as a daily

lunch pack for six months. Thereafter, these two groups of children were subjected to malaria parasite test every two months for six months using thick and thin blood films stained with 10% giemsa and examined under oil immersion objective lens. This was to determine the frequency of malaria parasite re-infection on both those who were fed with jollof rice and *Moringa oleifera* leaf powder and those who were fed with jollof rice only.

3.4 Objective 3: Effect of *Moringa oleifera* leaf powder on the Nutritional status of the children

3.4.1 Anthropometric data collection

The measurements of weight, height and mid upper arm circumferences of the pupils were taken at the beginning of the study and every six months for six months. The measurements were taken as follows.

3.4.2 Weight Measurement

Weight of each child was measured to the nearest 0.1 kilogramme (kg) with each participant lightly dressed using a portable digital scale (Tanita HD 309, Creative Health Products, MI, USA). Each child was asked to stand with both feet at the centre of the scale. Care was taken to see that the child did not lean forward or held any other support nearby and the weight was read.

3.4.3 Height Measurement

Height of each child was measured to the nearest 0.1 centimeters (cm). Each child stood bare foot and upright against a mounted stadiometer. Each child stood with the feet flat together and against the wall, the legs straight, arms at sides and shoulders level and looking straight ahead with the line of sight parallel with the floor. The measurement was taken while the child stood with head, shoulders, buttocks, and heels touching the flat surface (wall). The flat headpiece was used to form a right angle with the wall and lowered until it firmly touches the crown of the head and the height was read.

3.4.4 Mid Upper Arm Circumference (MUAC) Measurement

The MUAC was measured to the nearest 0.1cm using a calibrated Shakir strip with the left arm hanging relaxed and taken midway between the tip of acromion and olecranon process as described by Amirsheybani *et al* (2001). The left arm of each child was bent to identify the olecranon process and acromium and marked with a pen. The mid-point between these two was

marked. With the arm hanging straight down, a MUAC tape was wrapped around the arm at the midpoint mark and the measurement taken.

3.4.5 Calculation of Body Mass Index (BMI)

Body mass index (BMI) was used to determine the nutritional status of the children. BMI is a measure of body fat based on weight in relation to height. BMI was used as a screening tool to indicate whether the pupils were underweight, overweight, obese or healthy weight for their height. The BMI is defined as the body mass divided by the square of the body height, and is universally expressed in units of kg/m^2, resulting from mass in kilograms and height in metres. Commonly accepted BMI ranges are underweight: under 18.5 kg/m^2, normal weight: 18.5 to 25, overweight: 25 to 30, obese: over 30 (Malcolm, 2015). The weight and height of the pupils were measured using the standard protocols and the formula below applied to obtain the body mass index.

$$\text{Body Mass Index} = \frac{\text{Weight (in kilograms)}}{\text{Height (in meters)}^2}$$

3.5 Objective 4: Effects of *Moringa oleifera* leaf powder on the haematological profiles of the children

The hematological parameters viz packed cell volume, haemoglobin, white blood cell count, differentials (neutrophils, eosinophil, monocytes and lymphocytes), platelets, protein and iron were tested for each of the 500 selected children at the beginning of the study and every two months at three intervals into the study following feeding with jollof rice with or without *Moringa oleifera* leaf powder.

3.5.1 Packed cell volume (P.C.V.) Estimation

Packed cell volume (haematocrit) of each child was determined using capillary tubes and haematocrit centrifuge (Cheesbrough, 2000). The principle behind packed cell volume is to have whole blood centrifuged for maximum red blood cell packing. The space occupied by the red blood cell was measured and expressed as a percentage of the whole blood volume. A well mixed anticoagulated blood was allowed to enter into the special capillary tube until approximately ¾ filled with blood. The end of each haematocrit tube was sealed with plasticine.

The filled capillary tubes were placed in the grooves of each haematocrit centrifuge head. The sealed end was placed away from the centre of the centrifuge and covered by screwing up the lid adequately and centrifuged for 5 minutes at 3000 rotations per minute (rpm). The haematocrit tube was removed as soon as the centrifuge stopped spinning and the red cell column was read on the haematocrit tube reader and the formula below applied.

$$\text{PCV \%} = \frac{\text{Length of red cell column (mm)}}{\text{Length of total column (mm)}} \times 100$$

Normal range: 31 – 44%.

3.5.2 Haemoglobin (Hb) Estimation

This was accomplished using cymethaemoglobin method according to Baker *et al,* (1985). Four milliliter (4mls) of Drabkins solution was dispensed into a 75x10mm test tube using volumetric pipette. Twenty microlitres (20µl) of blood was dispensed into the same tube using haemoglobin micropipette. The contents of the tube were mixed and allowed to stand for 10 minutes at room temperature. The specthrophotometer was blanked with Drabkin's solution. The test and standard was calculated thus:

$$\frac{\text{Absorbance of test} \times \text{concentration of standard}}{\text{Absorbance of standard}} = \text{Hemoglobin in (g/dl)}$$

Normal range: 11.5-16.0g/dl.

3.5.3 Determination of White blood cell count

A thin film was prepared from the blood collected from each pupil and examined microscopically under X100 oil immersion objective lens. The smear was scanned to check for cell distribution, clumping, and abnormal cells. The peripheral edge of the smear was examined. The count began in the thin area of the slide. The white cell count was estimated by counting the number of WBCs by mathematical formula as follows:

WBC estimate = Total number of leukocytes counted X 3000 =? /µLS

Normal range: 4,500 – 11,000 cells/mcL (4.5-11.0x10^9/L

3.5.4 Determination of White blood cell differentials

White blood cell Differentials determines the percentage of each type of the mature white blood cells (monocytes, lymphocytes, neutrophils and eosinophils). A thin film of blood was prepared

from the blood collected from each pupil. The slides were allowed to adequately air dry and dipped in Wright's Stain for 10 seconds, dipped in distilled water for 20 seconds and air dried. The slides were examined microscopically for the different white blood cells under x10 objective lens for overall impression and general appearance of blood cells and under x100 objective lens after smearing oil droplet over length of slide. White blood cell differential was estimated by noting the number of different white cells per high power field X 100. Normal ranges of white blood cell differentials are as follows:

Neutrophils	2.0–7.0×10 ^{9}/l (40–80%)
Lymphocytes	1.0–3.0×10 ^{9}/l (20–50%)
Monocytes	0.2–1.0×10 ^{9}/l (2–10%)
Eosinophils	0.02–0.5×10 ^{9}/l (1–6%)

3.5.5 Platelet count Estimation

A thin film was prepared from the blood collected from each pupil on a grease free clean slide and timer set for 10 minutes. The smears were examined microscopically using the x100 oil objective (high powered field). The area of the smear where ½ the Red Blood Cells (RBCs) are overlapping and ½ are not overlapping was identified and the number of platelets counted on 5 successive fields, and the formula below applied:

$$\textbf{Platelet estimate} = \frac{\text{Total number of platelets counted}}{5} \times 15 = ?\ \underline{\text{X } 10^{3}/\mu\text{L}}$$

Normal range: 150,000 to 450,000/mcL.

3.5.6 Determination of Iron level

The blood samples in the plain tube were separated into clotted red cells and serum using Hermle microprocessor controlled bench centrifuge at 3000 rpm (rotations per minute). The serum was transferred into separate plain tubes using disposable plastic bulb pipettes. The method was according to Henry (1984). The test tubes were labeled blank, standard, control and sample. Into each of the tubes, 2.5mls of iron buffer reagent was added. Into each of the tubes, 0.5ml (500µl) of sample was added and mixed while 500µl iron free water was added to the blank tube. The blank tube was zeroed at 560nm spectrophotometer. The tubes were read and recorded for

absorbance (Ai reading). Into all the tubes, 0.5ml (50 µl) of iron colour reagent was added and mixed. The tubes were placed in the heating bath at 37°C for 10 minutes. The reagent blank was zeroed at 560nm (wavelength range: 520-560nm). The absorbance of all the tubes was read and recorded (A_2 reading).

Calculation: $\frac{A_2 \text{ Test} - A_1 \text{ Test}}{A_2 \text{ standard} - A_1 \text{ standard}}$ x conc. of standard= Total iron (ug/dl)

Normal range: 55 to 160µg/dl.

3.5.7 Determination of Protein level

The blood samples in the plain tube were separated into clotted red cells and serum using Hermle microprocessor controlled bench centrifuge at 3000 rpm (rotations per minute). The serum was transferred into separate plain tubes using disposables plastic bulb pipettes. The method was according to Tietz (1995). A new Gain Calibration with a cuvette containing fresh ddH_20 was performed. The test tubes was labeled reagent blank (S0), standard (S1), sample and sample blank. To the reagent blank, 0.01ml of distilled water was added, 0.01ml of standard (CAL) to standard (S1), 0.01ml of serum to sample and sample blank, 0.5ml of R1 (reagent 1) to reagent blank, standard and sample and 0.5ml of R2 (reagent 2) to sample blank. The contents in the tubes were mixed, incubated for 30mins at $+20^0$C to $+25^0$C. The absorbance of sample (A sample) and Standard (A standard) were measured against the reagent blank.

Calculation

Total protein Conc. = $\frac{\text{A sample}}{\text{A standard}}$ x Standard conc.

Normal range: 6 to 8.3g/dl (60-80g/l).

Data Analysis

Data collected were analyzed using descriptive statistics. Variations between groups were determined using chi-square and one way Anova of Statistical Package for Social Sciences (SPSS) version 20.

CHAPTER FOUR

RESULTS

4.1 Objective 1: The prevalence and intensity of malaria infection among children aged 4 to 5 years in Oba community

A total of 500 children aged 4-5 years 217 (43.4%) males and 283 (56.6%) females were tested for malaria parasite infection and 71(14.2%) were positive for malaria parasites while 429 (85.8%) tested negative (Table 1). This provided a baseline prevalence of 14.2% among the pupils. More females 42 (14.8%) than the males 29 (13.4%) were positive for malaria parasites but the difference was not significant (χ^2 cal = 0.22, χ^2 tab = 0.639, ($p>0.05$).

Table 1: Prevalence of malaria infection among children 4 to 5 years in Oba community by sex

Sex	No. Examined	No. Infected (%)	Negative (%)
Male	217	29 (13.4)	188 (86.6)
Female	283	42 (14.8)	241 (85.2)
Total	500	71(14.2)	429 (85.8)

χ^2 cal = 0.22, χ^2 tab = 0.639, ($p>0.05$)

4.1.2 Intensity of malaria infection among children 4 to 5 years in Oba community

There was a low intensity of malaria infection among the children (Table 2). Both the infected male pupils 29 (13.4%) and the infected female pupils 42 (14.8%) recorded below 1000 parasites per c.m.m which is a low intensity of infection.

Table 2: Intensity of malaria infection among children 4 to 5 years in Oba community

Sex	No. Examined	No infected (%)	Range of parasites per c.m.m Below 1000	1000 -10,000	10,000 -100,000	Over 100,000
Male	217	29 (13.4)	29 (13.4 %)	Nil	Nil	Nil
Female	283	42 (14.8)	42 (14.8 %)	Nil	Nil	Nil
Total	500	71 (14.2)	71 (14.2 %)	Nil	Nil	Nil

Sample size – 500 No. Negative = 429 or 85.8 %

4.2 Objective 2: The frequency of malaria parasite re-infection among children fed with jollof rice and *Moringa oleifera* leaf powder and those fed with jollof rice only at bimonthly intervals for six months.

At baseline, a malaria prevalence of 36 (14.4%) was recorded among the group fed with jollof rice and *Moringa oleifera* leaf powder, while for those fed with jollof rice only, a prevalence of 35 (14.0 %) was observed (Table 3). The infected children of both groups were treated with artemether lumefantrine and reintegrated into the study. At month two following feeding, the children fed with jollof rice and *Moringa oleifera* leaf powder did not test positive for malaria parasite while a significant prevalence of 46 (18.4%) was observed among the group fed with jollof rice only (χ^2 cal = 50.661, χ^2 tab = 0.000, $P<0.05$). They were treated with artemether-lumefantrine (coartem) and reintegrated into the study. At month four following feeding, both groups fed with jollof rice and *Moringa oleifera* leaf powder and the group fed with jollof rice only did not test positive for malaria parasite. Futhermore, at month six following feeding, none of the children fed with jollof rice and *Moringa oleifera* leaf powder was reinfected with malaria parasite but a significant prevalence of 36 (14.4%) was observed among the children fed with jollof rice only (χ^2 cal = 38.793, χ^2 tab = 0.000, $P<0.05$). The infected children were also treated with artemether-lumefantrine (coartem).

Table 3: Frequency of malaria reinfection in children 4-5 years old fed on jollof rice with *Moringa oleifera* leaf powder and those fed on jollof rice only at baseline and bimonthly intervals following feeding for six months

Period of study	Children fed with jollof rice and *Moringa oleifera* leaf powder		Children fed with jollof rice only	
	No examined	No Infected (%)	No examined	No Infected (%)
Baseline	250	36 (14.4)	250	35 (14.0)
2nd month following feeding	250	0 (0)	250	46 (18.4)
4th month following feeding	250	0 (0)	250	0 (0)
6th month following feeding	250	0 (0)	250	36 (14.4)

2nd month following feeding: χ^2 cal = 50.661, χ^2 tab = 0.000, $P<0.05$

6th month following feeding: χ^2 cal = 38.793, χ^2 tab = 0.000, $P<0.05$

4.2.2 Intensity of malaria parasite reinfection in children 4–5 years old fed with jollof rice and *Moringa oleifera* leaf powder and those fed with jollof rice only at bimonthly intervals following feeding for six months.

The results of parasite reinfection and intensity were shown in Table 4. At month two following feeding , the children fed with jollof rice and *Moringa oleifera* leaf powder had no malaria parasite re-infection and hence nil parasite intensity was observed while the children fed with jollof rice only had malaria parasite prevalence of 46 (18.4%) and a low parasite intensity of below 1000 parasites per cubic millimeter. At month four following feeding, both the children fed with jollof rice and *Moringa oleifera* leaf powder and those fed with jollof rice only were not re-infected with malaria parasite and no parasite intensity was observed. At month six following feeding the children fed with jollof rice and *Moringa oleifera* leaf powder had no malaria parasite re-infection and nil intensity of infection whereas those fed with jollof rice only were re-infected with a prevalence of 36 (14.4%) and low parasite intensity of below 1000 parasites per cubic millimeter. Thus there is a significant difference in parasite intensity among those fed with jollof rice and *Moringa oleifera* leaf powder and the group fed with jollof rice only (χ^2 cal = 500.1, χ^2 tab = 0.000, $P<0.05$).

Table 4: Intensity of malaria parasite reinfection in children 4–5 years old fed with jollof rice and *Moringa oleifera* leaf powder and those fed with jollof rice only at bimonthly intervals following feeding for six months

	Children fed on Jollof rice with *Moringa oleifera* leaf powder			Children fed on jollof rice only		
Period of study	No examined	No infected %	Range of parasite per c.m.m	No examined	No infected %	Range of parasite per c. m.m
2nd month following feeding	250	0 (0)	Nil	250	46 (18.4)	Below 1000
4th month following feeding	250	0 (0)	Nil	250	0 (0)	nil
6th month following feeding	250	0 (0)	Nil	250	36 (14.4)	Below 1000

χ^2 cal = 500.1, χ^2 tab = 0.000, P<0.05

Key

Source of variation (parasite intensity)	Range of parasite per c.m.m
+ (1-10) parasites per 100 high power field	Below 1,000
++ (11-100) parasites per 100 high power field	1,000-10,000
+++ (1-10) parasites in every high power field	10,000-100,000
++++ (more than 10) parasites in every high power field	Over 100,000

4.3 Objective 3: Effect of *Moringa oleifera leaf* powder on the nutritional status of the children.

4.3.1 Effect of *Moringa oleifera* leaf powder on the Weight of the children

The result in Table 5 showed that at baseline, the mean weight was 20.11kg $\pm$ 2.39 and 18.5kg $\pm$ 2.68 respectively for the children fed on jollof rice with *Moringa oleifera leaf* powder and those fed on jollof rice only. At month two following feeding, mean weight was 20.78kg $\pm$ 2.46 and 18.33kg $\pm$ 2.72 respectively for the children fed on jollof rice with *Moringa oleifera leaf* powder and those fed on jollof rice only. At month four following feeding, the mean weight was 20.90kg $\pm$ 2.48 and 18.32kg $\pm$ 2.69 respectively for the children fed on jollof rice with *Moringa oleifera leaf* powder and those fed on jollof rice only. Finally at month six following feeding, the mean weight was 21.14kg $\pm$ 2.62 and 18.41kg $\pm$ 2.65 respectively for the children fed on jollof rice with *Moringa oleifera leaf* powder and those fed on jollof rice only. Thus the baseline and bi-monthly weight measurements of the children fed on jollof rice with *Moringa oleifera leaf* powder was compared using Analysis of Variance (One –way ANOVA), an F-value of 7.696 with a p-value of 0.000 was obtained showing a significant improvement ($p<0.05$). Also the baseline and bi-monthly weight measurements of the children fed on jollof rice only was compared using Analysis of Variance (One –way ANOVA), an F-value of 0.257 with a p-value of 0.856 was obtained but the difference was not significant ($p>0.05$). Thus there is a significant effect of *Moringa oleifera* leaf powder on the weight of the children.

Table 5: Effect of *Moringa oleifera* leaf powder on the Weight of the children N=500

	Children fed on Jollof rice with *Moringa oleifera* leaf powder			Children fed on Jollof rice only	
Month	N	Mean Weight (kg)	Std deviation	Mean Weight (kg)	Std deviation
Baseline	250	20.11	2.38	18.5	2.67
2nd month	250	20.78	2.45	18.33	2.71
4th month	250	20.90	2.48	18.32	2.69
6th month	250	21.14	2.62	18.41	2.65

F-value 7.696 P-value 0.000 F-value 0.257 P-value 0.856

4.3.2 Effect of *Moringa oleifera* leaf powder on the Height of the children

The result in Table 6 showed that at baseline the mean height was 116.49cm $\pm$ 5.729 and 109.55cm $\pm$ 6.05 respectively for the children fed on jollof rice with *Moringa oleifera* leaf powder and those fed on jollof rice only. At month two following feeding, mean height was 116.81cm $\pm$ 5.83 and 109.59cm $\pm$ 6.10 respectively for the children fed on jollof rice with *Moringa oleifera* leaf powder and those fed on jollof rice only. At month four following feeding, mean height was 116.86cm $\pm$ 5.75 and 109.55cm $\pm$ 6.09 respectively for the children fed on jollof rice with *Moringa oleifera* leaf powder and those fed on jollof rice only. Finally at month six following feeding, mean height was 116.94cm $\pm$ 5.80 and 109.64cm $\pm$ 6.14 respectively for the children fed on jollof rice with *Moringa oleifera* leaf powder and those fed on jollof rice only. The baseline and bi-monthly heights of the children fed on jollof rice with *Moringa oleifera* leaf powder was compared using Analysis of Variance (One –way ANOVA), an F-value of 0.298 with a p-value of 0.827 was obtained but the difference was not significant ($p>0.05$). The baseline and bi-monthly heights of the children fed on jollof rice only was also compared using Analysis of Variance (One –way ANOVA), an F-value of 0.012 and a p-value of 0.298 was obtained and the difference was also not significant ($p>0.05$).

Table 6: Effect of *Moringa oleifera* leaf powder on the Height of the children N=500

Children fed on Jollof rice with *Moringa oleifera* leaf powder				Children fed on Jollof rice only	
Month	N	Mean height (cm)	Standard deviation	Mean height (cm)	Standard deviation
Baseline	250	116.4	5.72	109.55	6.05
2nd month	250	116.81	5.83	109.55	6.10
4th month	250	116.86	5.75	109.59	6.09
6th month	250	116.94	5.80	109.64	6.14

F-value 0.298 P-value 0.827 F-value 0.012 P-value 0.298

4.3.3 Effect of *Moringa oleifera* leaf powder on the Mid Upper Arm Circumference (MUAC) of the children

The results in Table 7 showed that at baseline, the mean mid upper arm circumference was 15.72cm ± 1.10 and 15.40cm ± 0.87 respectively for the children fed on jollof rice with *Moringa oleifera* leaf powder and those fed on jollof rice only. At month two following feeding, mean mid upper arm cirdumference was 16.31cm ± 1.14 and 15.54cm ± 0.96 respectively for the children fed on jollof rice with *Moringa oleifera* leaf powder and those fed on jollof rice only. At month four following feeding, mean mid upper arm circumference was 16.38cm ± 1.09 and 15.54cm ± 0.95 respectively for the children fed on jollof rice with *Moringa oleifera* leaf powder and those fed on jollof rice only. Finally at month six following feeding, mean mid upper arm circumference was 16.59cm ± 1.43 and 15.60cm ± 0.97 respectively for the children fed on jollof rice with *Moringa oleifera* leaf powder and those fed on jollof rice only. The baseline and bi-monthly mid upper arm circumference of the children fed on jollof rice with *Moringa oleifera* leaf powder were compared using Analysis of Variance (One –way ANOVA), an F-value of 24.082 and a p-value of 0.000 was obtained $p<0.05$ showing a highly significant improvement. The baseline and bi-monthly mid upper arm circumference of the children fed on jollof rice only were also compared using Analysis of Variance (One –way ANOVA), an F-value of 2.05 and p-value of 0.105 was obtained $p<0.05$ (slightly significant).

Table 7: Effect of *Moringa oleifera* leaf powder on the Mid Upper Arm Circumference (MUAC) of the children=500

		Children fed on Jollof rice with *Moringa oleifera* leaf powder		Children fed on Jollof rice only	
Month	N	Mean MUAC (cm)	Standard deviation	Mean MUAC in cm	Standard deviation
Baseline	250	15.72	1.10	15.40	0.87
2nd month	250	16.31	1.14	15.54	0.96
4th month	250	16.38	1.09	15.54	0.95
6th month	250	16.59	1.43	15.60	0.97

F-value 24.082 P-value 0.000 F-value 2.05 P-value 0.105

4.3.4 Body Mass Index of children 4-5 years old fed on jollof rice with *Moringa oleifera leaf* powder and those fed on jollof rice only

The result in Table 8 showed that at baseline the body mass indices (BMI) of the children fed on jollof rice with *Moringa oleifera leaf* powder were 84 (33.6%) under weight, 137 (54.8%) healthy weight, 20 (8%) overweight and 9 (3.6%) obese while among those fed on jollof rice only BMI were 76 (30.4%) underweight, 149 (59.6%) healthy weight, 14 (5.6%) overweight and 11 (4.4%) obese. At month two following feeding, among the children fed on jollof rice with *Moringa oleifera leaf* powder underweight children reduced to 64 (25.6%) while healthy weight, overweight and obese increased to 151 (60.4%), 23 (9.2%) and 12 (4.8%) respectively. Among those fed on jollof rice only, underweight increased to 83 (33.2%) and a mild reduction in the numbers of healthy weight, overweight and obese of 144 (57.6%), 13 (5.2%) and 11 (4%) respectively were observed. At month four following feeding among those fed on jollof rice with *Moringa oleifera leaf* powder underweight further reduced to 39 (15.6%) while healthy weight, over weight and obese increased to 169 (67.6%), 24 (9.6%) and 18 (7.2%) respectively. However, for those fed on jollof rice only, there was also a reduction in underweight of 65 (26%) while an increase in healthy weight, over weight and obese of 156 (62.4%), 17(6.8%) and 12 (4.8%) respectively were observed. Finally at the sixth month following feeding, those fed on jollof rice with *Moringa oleifera leaf* powder had a reduced underweight of 27 (10.8%) while there was an increase in healthy weight, over weight and obese of 179 (71.6%), 26 (10.4%) and 18 (7.2%) respectively while for the children fed on jollof rice only, underweight further increased to 85 (34%) while healthy weight, over weight and obese reduced to 149 (59.6%), 12 (4.8%) and 4 (1.6%) respectively. A chi-square value of 2.721 and a p-value of 0.257 were obtained. Thus the observed increase in the number of children having healthy weight, overweight and obese among the children fed on jollof rice with *Moringa oleifera leaf* powder when compared with the chidren fed on jollof rice only shows that *Moringa oleifera leaf* powder has a positive effect on the nutritional status of the children.

Table 8: Body Mass Index of children 4-5 years old fed on jollof rice with *Moringa oleifera leaf* powder and those fed on jollof rice only

	Children fed on Jollof rice with *Moringa oleifera* leaf powder					Children fed on Jollof rice only			
Study period	No Examined	Under weight	Healthy Weight	Over weight	Obese	Under weight	Healthy weight	Over Weight	Obese
Baseline	250	84 (33.6%)	137 (54.8%)	20 (8%)	9 (3.6%)	76 (30.4%)	149 (59.6%)	14 (5.6%)	11 (4.4%)
2nd month following feeding	250	64 (25.6%)	151 (60.4%)	23 (9.2%)	12 (4.8%)	83 (33.2%)	144 (57.6%)	13 (5.2%)	10 (4%)
4th month following feeding	250	39 (15.6%)	169 (67.6%)	24 (9.6)%	18 (7.2%)	65 (26%)	156 (62.4%)	17 (6.8%)	12 (4.8%)
6th month following feeding	250	27 (10.8%)	179 (71.6%)	26 (10.4%)	18 (7.2)	85 (34%)	149 (59.6%)	12 (4.8%)	4 (1.6%)

Chi-square 2.721 P-value 0.257

4.4 Objective 4: Effect of *Moringa oleifera* leaf powder on the haematological parameters such as packed cell volume, haemoglobin, white blood cell count (Neutrophils, lymphocytes, Monocytes and Eosinophils), Platelets, iron and protein of children 4-5years in Oba community.

4.4.1 Effect of *Moringa oleifera* leaf powder on the packed cell volume (PCV) of the children

At baseline, the mean packed cell volume was 30% $\pm$ 0.02 and 29% $\pm$ 0.01 respectively for the children fed on jollof rice with *Moringa oleifera* leaf powder and those fed on jollof rice only as (Table 9). At month two following feeding, the mean packed cell volume was 31% $\pm$ 0.02 and 29% $\pm$ 0.02 respectively for the children fed on jollof rice with *Moringa oleifera* leaf powder and those fed on jollof rice only. At month four following feeding, mean packed cell volume was 31% $\pm$ 0.02 and 29% $\pm$ 0.01 respectively for the children fed on jollof rice with *Moringa oleifera* leaf powder and those fed on jollof rice only. Finally at month six following feeding, mean Packed cell volume was 32% $\pm$ 0.02 and 29% $\pm$ 0.01 respectively for the children fed on jollof rice with *Moringa oleifera* leaf powder and those fed on jollof rice only. The baseline and bi-monthly packed cell volume of the children fed on jollof rice with *Moringa oleifera* leaf powder was compared using Analysis of Variance (One –way ANOVA), an F-value of 36.496 with a p-value of 0.000 was obtained and the difference is highly significant ($p<0.05$). Also the baseline and bi-monthly packed cell volume of the children fed on jollof rice only was compared using Analysis of Variance (One –way ANOVA), an F-value of 0.478 with a p value of 0.830 was obtained with no significant difference.

Table 9: Effect of *Moringa oleifera* leaf powder on the packed cell volume (PCV) of the children

N=500

Children fed on Jollof rice with *Moringa oleifera* leaf powder				Children fed on Jollof rice only	
Month	N	Mean PCV (%)	Standard deviation	Mean PCV (%)	Standard deviation
Baseline	250	30	0.02	29	0.01
2nd month	250	31	0.02	29	0.02
4th month	250	31	0.02	29	0.01
6th month	250	32	0.02	29	0.01

F-value 36.497 P-value 0.000 F-value 0.478 P-value 0.830

4.4.2 Effect of *Moringa oleifera* leaf powder on the Haemoglobin (HB) of children

Table 10 showed that mean haemoglobin at baseline was 10.08 g/dl $\pm$ 0.9 and 9.92 g/dl $\pm$ 0.63 respectively for the children fed on jollof rice with *Moringa oleifera* leaf powder and those fed on jollof rice only. At month two following feeding, mean haemoglobin was 10.39 g/dl $\pm$ 0.69 and 9.87 g/dl $\pm$ 0.70 respectively for the children fed on jollof rice with *Moringa oleifera* leaf powder and those fed on jollof rice only. At month four following feeding, mean haemoglobin was 10.47 g/dl $\pm$ .68 and 9.91 g/dl $\pm$ 0.63 respectively for the children fed on jollof rice with *Moringa oleifera* leaf powder and those fed on jollof rice only. Finally at month six following feeding, mean haemoglobin was 10.78 g/dl $\pm$ 0.70 and 9.96 g/dl $\pm$ 0.59 respectively for the children fed on jollof rice with *Moringa oleifera* leaf powder and those fed on jollof rice only. The baseline and bi-monthly haemoglobin of the children fed on jollof rice with *Moringa oleifera* leaf powder was compared using Analysis of Variance (One –way ANOVA), an F-value of 36.539 and p-value of 0.000 was obtained showing a significant difference ($p<0.05$). The baseline and bi-monthly haemoglobin of the children fed on jollof rice only was also compared using Analysis of Variance (One –way ANOVA), an F-value of 0.477 and p-value of 0.832 was obtained and the difference was not significant ($p>0.05$).

Table 10: Effect of *Moringa oleifera* leaf powder on the Haemoglobin (Hb) of children

N=500

	Children fed on Jollof rice with *Moringa oleifera* leaf powder			Children fed on Jollof rice only	
Month	N	Mean HB (g/dl)	Standard deviation	Mean HB (g/dl)	Standard deviation
Baseline	250	10.08	0.90	9.92	0.63
2nd month	250	10.39	0.69	9.87	0.70
4th month	250	10.47	0.68	9.91	0.63
6th month	250	10.78	0.70	9.96	0.59

F-value 36.539 P-value 0.000 F-value 0.477 P-value 0.832

4.4.3 Effect of *Moringa oleifera* leaf powder on the White Blood Cell (WBC) of children

At baseline (Table 11), the mean white blood cell count was 5.88 $x10^9/l \pm 1.05$ and 5.68 $x10^9/l \pm 0.82$ respectively for the children fed on jollof rice with *Moringa oleifera* leaf powder and those fed on jollof rice only. At month two following feeding, mean white blood cell count was 5.99 $x10^9/l \pm 1.03$ and 5.68 $x10^9/l \pm 0.82$ respectively for the children fed on jollof rice with *Moringa oleifera* leaf powder and those fed on jollof rice only. At month four following feeding, mean white blood cell count was 6.06 $x10^9/l \pm 0.98$ and 5.70 $x10^9/l \pm 0.82$ respectively for the children fed on jollof rice with *Moringa oleifera* leaf powder and those fed on jollof rice only. Finally at month six following feeding, mean white blood cell count was 6.17 $x10^9/l \pm 0.87$ and 5.80 $x10^9/l \pm 0.85$ respectively for the children fed on jollof rice with *Moringa oleifera* leaf powder and those fed on jollof rice only. The baseline and bi-monthly white blood cell count of the children who were fed on jollof rice with *Moringa oleifera* leaf powder was compared using Analysis of Variance (One –way ANOVA). An F-value of 3.793 with a p-value of 0.010 was obtained with significant difference ($p<0.05$). The baseline and bi-monthly white blood cell count of the children fed on jollof rice only was compared using Analysis of Variance (One –way ANOVA) an F-value of 1.301 with a p-value of 0.273 was obtained and the difference was also significant ($p<0.05$).

Table 11: Effect of *Moringa oleifera* leaf powder on the White Blood Cell (WBC) of children

N=500

		Children fed on Jollof rice with *Moringa oleifera* leaf powder		Children fed on Jollof rice only	
Month	N	Mean WBC ($x10^9$/l)	Standard deviation	Mean WBC ($x10^9$/l)	Standard deviation
Baseline	250	5.88	1.05	5.68	0.82
2nd month	250	5.99	1.03	5.68	0.82
4th month	250	6.06	0.98	5.70	0.82
6th month	250	6.17	0.87	5.80	0.85

F-value 3.793 P-value 0.010 F-value 1.301 P-value 0.273

4.4.4 Effect of *Moringa oleifera* leaf powder on the Neutrophils of children

At baseline (Table 12) the mean Neutrophil was 43.92% ± 6.24 and 46.67% ± 6.94 respectively for the children fed on jollof rice with *Moringa oleifera* leaf powder and those fed on jollof rice only. At month two following feeding, mean Neutrophil was 45.08% ± 6.1 and 46.83% ± 6.50 respectively for the children fed on jollof rice with *Moringa oleifera* leaf powder and those fed on jollof rice only. At month four following feeding, mean Neutrophil was 45.92% ± 6.37 and 46.82% ± 6.55 respectively for the children fed on jollof rice with *Moringa oleifera* leaf powder and those fed on jollof rice only. Finally at the sixth month following feeding, the mean Neutrophil was 39.35% ± 15.83 and 46.52% ± 7.15 respectively for the children fed on jollof rice with *Moringa oleifera* leaf powder and those fed on jollof rice only. The baseline and bi-monthly Neutrophil of the children who were fed on jollof rice with *Moringa oleifera* leaf powder were compared using Analysis of Variance (One –way ANOVA), an F-value of 23.302 with a p-value of 0.000 was obtained showing a significant reduction ($p<0.05$). The baseline and bi-monthly Neutrophil of the group fed on jollof rice only was compared using Analysis of Variance (One –way ANOVA), an F-value of 0.118 and a p-value of 0.950 was obtained but the difference was not significant ($p>0.05$).

Table 12: Effect of *Moringa oleifera* leaf powder on the Neutrophil of children

N=500

		Children fed on Jollof rice with *Moringa oleifera* leaf powder		Children fed on Jollof rice only	
Month	N	Mean Neutrophil (%)	Standard deviation	Mean Neutrophil (%)	Standard deviation
Baseline	250	43.92	6.24	46.67	6.94
2nd month	250	45.08	6.10	46.83	6.50
4th month	250	45.92	6.37	46.82	6.55
6th month	250	39.30	15.80	46.52	7.15

F-value 23.302 P-value 0.000 F-value 0.118 P-value 0.950

4.4.5 Effect of *Moringa oleifera* leaf powder on the lymphocyte of children

At baseline (Table 13), the mean lymphocyte was 48.84% ± 51.25 and 48.10% ± 7.30 respectively for the children fed on jollof rice with *Moringa oleifera* leaf powder and those fed on jollof rice only. At month two following feeding, the mean lymphocyte was 50.04% ± 51.25 and 48.22% ± 7.30 respectively for the children fed on jollof rice with *Moringa oleifera* leaf powder and those fed on jollof rice only. At month four following feeding, the mean lymphocyte was 49.40% ± 11.12 and 47.01% ± 10.69 respectively for the children fed on jollof rice with *Moringa oleifera* leaf powder and those fed on jollof rice only. Finally at month six following feeding, the mean lymphocyte was 51.25% ± 6.44 and 48.68% ± 7.61 respectively for the children fed on jollof rice with *Moringa oleifera* leaf powder and those fed on jollof rice only. The baseline and bi-monthly lymphocyte of the children fed on jollof rice with *Moringa oleifera* leaf powder was compared using Analysis of Variance (One –way ANOVA); an F-value of 4.387 with a p-value of 0.004 was obtained showing a significant improvement ($p<0.05$). The baseline and bi-monthly lymphocyte of the children fed on jollof rice only was also compared using Analysis of Variance (One –way ANOVA); an F-value of 1.792 with a p-value of 0.147 was obtained showing a slight significant difference ($p<0.05$).

Table 13: Effect of *Moringa oleifera* leaf powder on the lymphocyte of children

N=500

	Children fed on Jollof rice with *Moringa oleifera* leaf powder			Children fed on Jollof rice only	
Month	N	Mean lymphocyte (%)	Standard deviation	Mean lymphocyte (%)	Standard deviation
Baseline	250	48.84	51.25	48.10	7.30
2nd month	250	50.04	51.25	48.22	7.30
4th month	250	49.4	11.12	47.01	10.69
6th month	250	51.25	6.44	48.68	7.61

F-value 4.387 P-value 0.004 F-value 1.792 P-value 0.147

4.4.6 Effect of *Moringa oleifera* leaf powder on the Monocyte of children

At baseline (Table 14), the mean Monocyte was 2.17% ± 1.22 and 2.18% ± 1.07 respectively for the children fed on jollof rice with *Moringa oleifera* leaf powder and those fed on jollof rice only. At month two following feeding, the mean Monocyte was 2.66 % ± 1.48 and 2.23 % ± 1.09 respectively for the children fed on jollof rice with *Moringa oleifera* leaf powder and those fed on jollof rice only. At month four following feeding, the mean Monocyte was 2.96 % ± 1.2 and 2.3 % ± 1.04 respectively for the children fed on jollof rice with *Moringa oleifera* leaf powder and those fed on jollof rice only. Finally at month six following feeding, the mean Monocyte was 3.21% ± 1.17 and 2.52 % ± 0.89 respectively for the children fed on jollof rice with *Moringa oleifera* leaf powder and those fed on jollof rice only. The baseline and bi-monthly Monocyte of the children fed on jollof rice with *Moringa oleifera* leaf powder was compared using Analysis of Variance (One –way ANOVA), an F-value of 30.449 and a p-value of 0.000 was obtained showing a highly significant improvement ($p<0.05$). The baseline and bi-monthly Monocyte of the children fed on jollof rice only was also compared using Analysis of Variance (One –way ANOVA), an F-value of 5.430 and a p-value of 0.001 was obtained and the difference was also significant ($p<0.05$)

Table 14: Effect of *Moringa oleifera* leaf powder on the Monocyte of children

N=500

		Children fed on Jollof rice with *Moringa oleifera* leaf powder		Children fed on Jollof rice only	
Month	N	Mean Monocyte (%)	Standard deviation	Mean Monocyte (%)	Standard deviation
Baseline	250	2.17	1.22	2.18	1.07
2nd month	250	2.66	1.48	2.23	1.09
4th month	250	2.96	1.20	2.30	1.04
6th month	250	3.21	1.17	2.52	0.89

F-value 30.449 P-value 0.000 F-value 5.430 P-value 0.001

4.4.7 Effect of *Moringa oleifera* leaf powder on the Eosinophil of children

The results in Table 15 showed that at baseline, the mean Eosinophil was 2.31% ± 1.31 and 2.78% ± 1.15 respectively for the children fed on jollof rice with *Moringa oleifera* leaf powder and those fed on jollof rice only. At month two following feeding, the mean Eosinophil was 2.94 % ± 1.49 and 2.78 % ± 1.09 respectively for the children fed on jollof rice with *Moringa oleifera* leaf powder and those fed on jollof rice only. At month four following feeding, the mean Eosinophil was 3.16 % ± 1.43 and 2.80 % ± 1.01 respectively for those fed on jollof rice with *Moringa oleifera* leaf powder and those fed on jollof rice only. Finally at month six following feeding, the mean Eosinophil was 3.32 % ± 1.31 and 2.99 % ± .93 respectively for the children fed on jollof rice with *Moringa oleifera* leaf powder and those fed on jollof rice only. The baseline and bi-monthly Eosinophil of the children fed on jollof rice with *Moringa oleifera* leaf powder was compared using Analysis of Variance (One –way ANOVA), an F-value of 25.663 and p-value of 0.000 was obtained showing a highly significant improvement ($p<0.05$). The baseline and bi-monthly Eosinophil of the children fed on jollof rice only was also compared using Analysis of Variance (One –way ANOVA), an F-value of 2.405 and p-value of 0.066 was obtained with significant difference ($p<0.05$).

Table 15: Effect of *Moringa oleifera* leaf powder on the Eosinophil of children

N=500

		Children fed on Jollof rice with *Moringa oleifera* leaf powder		Children fed on Jollof rice only	
Month	N	Mean Eosinophil (%)	Standard deviation	Mean Eosinophil (%)	Standard deviation
Baseline	250	2.31	1.31	2.78	1.15
2nd month	250	2.94	1.49	2.78	1.09
4th month	250	3.16	1.43	2.80	1.01
6th month	250	3.32	1.31	2.99	0.93

F-value 25.663 P-value 0.000 F-value 2.405 P-value 0.066

4.4.8 Effect of *Moringa oleifera* leaf powder on the Platelets of children

The results in Table 16 showed that at baseline the mean platelet was 273.17/mcl ± 65.2 and 279.15/mcl ± 66.72 respectively for the children fed on jollof rice with *Moringa oleifera* leaf powder and those fed on jollof rice only. At month two following feeding, mean platelet was 277.48/mcl ± 65.65 and 277.61/mcl ± 68.98 respectively for the children fed on jollof rice with *Moringa oleifera* leaf powder and those fed on jollof rice only. At month four following feeding, mean platelet was 289.87/mcl ± 72.6 and 277.96/mcl ± 68.89 respectively for the children fed on jollof rice with *Moringa oleifera* leaf powder and those fed on jollof rice only. Finally at month six following feeding, mean platelet was 286.83/mcl ± 78.2 and 279.33/mcl ± 67.96 respectively for the children fed on jollof rice with *Moringa oleifera* leaf powder and those fed on jollof rice only. The baseline and bi-monthly platelet of the children fed on jollof rice with *Moringa oleifera* leaf powder was compared using Analysis of Variance (One –way ANOVA), an F-value of 3.070 with a p-value of 0.027 was obtained showing a significant improvement ($p<0.05$). The baseline and bi-monthly platelet of the children fed on jollof rice only was also compared using Analysis of Variance (One –way ANOVA), an F-value of 0.039 with a p-value of 0.990 was obtained but the difference was not significant ($p>0.05$).

Table 16: Effect of *Moringa oleifera* leaf powder on the Platelets of children

N=500

		Children fed on Jollof rice with *Moringa oleifera* leaf powder		Children fed on Jollof rice only	
Month	N	Mean Platelet/mcl	Standard deviation	Mean Platelet/mcl	Standard deviation
Baseline	250	273.17	65.20	279.15	66.72
2nd month	250	277.48	65.65	277.61	68.98
4th month	250	289.87	72.60	277.96	68.89
6th month	250	286.83	78.20	279.33	67.96

F-value 3.070 P-value 0.027 F-value 0.039 P-value 0.990

4.4.9 Effect of *Moringa oleifera* leaf powder on the Protein of children

The results in Table 17 show that at baseline mean protein was 68.74g/l ± 8.46 and 73.33g/l ± 6.89 respectively for the children fed on jollof rice with *Moringa oleifera* leaf powder and those fed on jollof rice only. At month two following feeding, mean protein was 71.20 g/l ± 8.15 and 73.12 g/l ± 6.96 respectively for the children fed on jollof rice with *Moringa oleifera* leaf powder and those fed on jollof rice only. At month four following feeding, mean protein was 74.72 g/l ± 7.8 and 73.25 g/l ± 7.06 respectively for the children fed on jollof rice with *Moringa oleifera* leaf powder and those fed on jollof rice only. Finally at month six following feeding, mean protein was 73.45 g/l ± 8 and 71.71 g/l ± 7.17 respectively for the children fed on jollof rice with *Moringa oleifera* leaf powder and those fed on jollof rice only. The baseline and bi-monthly protein of the children fed on jollof rice with *Moringa oleifera* leaf powder was compared using Analysis of Variance (One –way ANOVA), an F-value of 26.382 and a p-value of 0.000 was obtained showing a highly significant improvement ($p<0.05$). The baseline and bi-monthly protein of the children fed on jollof rice only was also compared using Analysis of Variance (One –way ANOVA), an F-value of 0.185 and a p-value of 0.907 was obtained but the difference was not significant ($p>0.05$).

Table 17: Effect of *Moringa oleifera* leaf powder on the Protein of children

N=500

		Children fed on Jollof rice with *Moringa oleifera* leaf powder		Children fed on Jollof rice only	
Month	N	Mean Protein (g/l)	Standard deviation	Mean Protein (g/l)	Standard deviation
Baseline	250	68.74	8.46	73.33	6.89
2nd month	250	71.20	8.15	73.12	6.96
4th month	250	74.72	7.8	73.25	7.06
6th month	250	73.45	8.00	71.71	7.17

F-value 26.382 P-value 0.000 F-value 0.185 P-value 0.907

4.4.10 Effect of *Moringa oleifera* leaf powder on the Iron of children

The results in Table 18 show that at baseline mean iron was 65.29µg/dl ± 19.13 and 74.63 µg/dl ± 13.07 respectively for the children fed on jollof rice with *Moringa oleifera* leaf powder and those fed on jollof rice only. At month two following feeding, mean iron was 71.12 µg/dl ± 17.71 and 74.52 µg/dl ± 13.2 respectively for the children fed on jollof rice with *Moringa oleifera* leaf powder and those fed on jollof rice only. At month four following feeding, mean iron was 75.70 µg/dl ± 17.84 and 74.32 µg/dl ± 13.4 respectively for the children fed on jollof rice with *Moringa oleifera* leaf powder and those fed on jollof rice only. Finally at month six following feeding, mean iron was 77.30 µg/dl ± 16.3 and 74.38 µg/dl ± 11.95 respectively for the children fed on jollof rice with *Moringa oleifera* leaf powder and those fed on jollof rice only. The baseline and bi-monthly iron of the children fed on jollof rice with *Moringa oleifera* leaf powder was compared using Analysis of Variance (One –way ANOVA), an F-value of 22.977 and a p-value of 0.000 was obtained showing a highly significant improvement ($p<0.05$). The baseline and bi-monthly iron of the children fed on jollof rice only was also compared using Analysis of Variance (One –way ANOVA), an F-value of 0.030 and p-value of 0.993 was obtained but the difference was not significant ($p>0.05$).

Table 18: Effect of *Moringa oleifera* leaf powder on the Iron of children

		Children fed on Jollof rice with *Moringa oleifera* leaf powder		Children fed on Jollof rice only	
Month	N	Mean Iron (µg/dl)	Standard deviation	Mean Iron (µg/dl)	Standard deviation
Baseline	250	65.29	19.13	74.63	13.07
2nd month	250	71.12	17.71	74.52	13.20
4th month	250	75.70	17.84	74.32	13.40
6th month	250	77.00	16.30	74.38	11.95

F-value 22.977 P-value 0.000 F-value 0.030 P-value 0.993

CHAPTER FIVE

DISCUSSION

5.1 Baseline Prevalence and Intensity

Of the 500 pupils whose blood samples were tested for malaria parasite at the beginning of the study, 71 (14.2%) were positive for malaria parasite with a parasite intensity of 3% and *P. falciparum* as the only species. The observed low prevalence agrees with the study of Ogolo *et al* (2015) who reported a prevalence rate of 10.3% among secondary school students' aged 16 years in Idemili south Anambra State, Ahmed *et al,* (2001) also reported a prevalence rate of 6% in Maiduguri North Eastern Nigeria.

However, this is in contrast with reports of Nwaorgu and Orajaka (2011) who recorded high prevalence rates of 71.2% in children 4 years old and 55.60% in children 5 years old in Awka North Local Government Area, Mbanugo and Emenalo, (2004) also recorded a high prevalence rate of 77.4% in Owerri and Ukpai and Ajolu, (2001) reported prevalence rates of 75% in Owerri and 85.5% in Okigwe.

The low prevalence may be due to the distribution of long lasting insecticidal treated bed nets in the community in 2014 by the Anambra State Ministry of Health. The use of bed nets might have reduced mosquito-man contact and reduced malaria parasite transmission. *P.falciparum* being the only species found is in line with other studies (Ogolo *et al,* 2015, Nwaorgu and Orajaka, 2011, Umeanaeto *et al*, 2006; Mbanugo and Emenalo, 2004).

The low parasite intensity also agrees with the study of Nwaorgu and Orajaka (2011) in which majority of children infected with malaria parasite have low parasite intensity below 1000per c.m.m. The low intensity indicates that the parasite load is low and may be the reason why the infected children still attend school.

5.2 Frequency of Malaria reinfection

On the frequency of malaria parasite, at month two following feeding, none of the children that were fed on jollof rice with *Moringa oleifera* leaf powder was reinfected while for those that were fed on jollof rice without *Moringa oleifera* leaf powder 46 (18.4%) were reinfected with parasite intensity of 3% and *P. falciparum* as the only species.

At month four following feeding, there was no malaria parasite reinfection on both the group fed on jollof rice with *Moringa oleifera* leaf powder and those fed on jollof rice only. The reason for

the absence of reinfection in both groups at month four following feeding may be because those positive during the second month were treated with an antimalaria drug artemether lumefantrine (coartem) which is in line with the study of Issaka *et al,* (2002) who noted a 7 to 9 weeks post treatment reinfection with malaria parasite. This contrasts with the findings of Woodring *et al* (2010) who observed a 37 days recurrent malaria parasitaemia in children after treatment with artemether-lumefantrine.

At month six following feeding, all the children fed on jollof rice with *Moringa oleifera* leaf powder were not infected with malaria parasite while for those that were fed on jollof rice only, 36 (14.4%) were reinfected with parasite intensity of 2% and *P. falciparum* as the only specie.

This finding supports the study of Olasehinde *et al* (2012) that the potency of crude *Moringa oleifera* extracts in mice is a confirmation of the rationale for its use in malaria treatment among indigenous Nigerians. It also supports the findings of Obasi and Mba (2010) that *Moringa oleifera* leave extract can effectively protect animal hosts against *Plasmodium* by preventing the establishment of new infections and progression of the disease into heavy attacks. The presence of malaria parasite infection in the group fed on jollof rice only between two to three months is in line with the study of Issaka *et al*, (2002) who noted a 7 to 9 weeks post treatment reinfection with malaria parasite and Murphy and Breman, (2001) who reported that African children have between 1.6 and 5.4 episodes of malarial fever each year.

The delayed parasitaemia in the group who were fed on jollof rice with *Moringa oleifera* leaf powder may be as a result of the fact that *Moringa oleifera* leaf contains both essential and non essential amino acids (Igwilo *et al,* 2011) and a very strong concentrations of Vitamin A, C, B complex, Iron, calcium, protein, Zinc and Selenium (Fuglie, 2001). Vitamin A is a potent anti-infective agent that assists animals and the human body to develop disease resistance (Anwar *et al*, 2007).

Moreover, it may also be as a result of the presence of phytochemicals such as phenol, flavonoids, tannin, saponin, terpenoids and alkaloids in *Moringa oleifera* leaf which has possible preventive and curative properties.

Flavonoids, phenol and tannin have antioxidant effects; they react with and capture dangerously reactive compounds called free radicals preventing the radicals from reacting with other biomolecules and causing serious damage in the body (Majumdar, 2014).

Saponins bind with cholesterol and pathogens in the body and prevent them from being absorbed by the body, carrying them through the body's digestive system to be eliminated. Saponins also acts as an antioxidant, get rid of destructive pathogens in the body thus relieving stress on the immune system for effective functioning (Josephine *et al*, 2010).

Terpenoids have a broad range of biological activities such as analgesic, anti-inflammatory, antimicrobial, antifungal, antiviral, and antiparasitic activities (Singh and Sharma, 2015). Alkaloids have a wide range of pharmacological activities including antimalarial (*e.g.* quinine), antiasthma (*e.g.* ephedrine), anticancer (*e.g.* homoharringtonine) (Kittakoop *et al*, 2014). The presence of these phytochemicals may have boosted the immunity of the children fed on jollof rice with *Moringa oleifera* leaf powder and contribute to the absence of malaria parasitaemia during the period of six months.

5.3 Effect of *Moringa oleifera* leaf powder on Nutritional status

On the effect of *Moringa oleifera* leaf powder on the nutritional status of the children, there was a significant improvement in the anthropometric indices of the children fed on jollof rice with *Moringa oleifera* leaf powder when compared with the group who were fed on only jollof rice. A significant increase in weight from 20.1kg to 21.1 kg was observed for the group fed on jollof rice with *Moringa oleifera* leaf powder as against 18.5kg to 18.4kg for those fed on only jollof rice after six months period. The increase in weight may be due to phythochemicals like phytic acid and tanin in *Moringa oleifera* leaf that increase the gut transit time and aid in more efficient absorption of nutrients (Muraoka ad Mura, 2004). It may also be due to its high concentration of vitamins that promotes heathy digestion and absorption (Fuglie, 2001).

There was no significant increase in height in both the group fed on jollof rice with *Moringa oleifera* leaf powder and the group fed on only jollof rice with 116.4cm to 116.9cm and 109.5cm to 109.6cm respectively over the six months period.

The mid upper arm circumference of the group who were fed on jollof rice with *Moringa oleifera* leaf powder increased from 15.7cm to 16.5cm showing a highly significant improvement while that of the group who were fed on only jollof rice increased from 15.4cm to 15.6cm with slight significant difference over six months.

This finding agrees with Ogbuagu *et al,* (2014) with regards to increase in weight from 30.5kg to 39.7 kg among children whose diets were supplemented with *Moringa oleifera* leaf powder in Anambra State. Urbain *et al,* (2013) in the study of malnourished children in Bukina Faso also

observed a weight gain of 8.9g/kg/day when the diet of children 6-59 months was supplemented with 10g of *Moringa oleifera* leaf powder and also Igwilo *et al,* (2011) observed an increase in weight in albino rats fed on *M. oleifera* leaf powder for three weeks from 46.73 to 66. 59 when compared with those fed on casilan diet from 55.94 to 42.89. Also in a study in India, Vanisha *et al,* (2003) noted an increase in anthropometric parameters of children whose diet was supplemented with 7g of *Moringa oleifera* leaf for one month.

The body mass index showed a reduction on the underweight children over six months from 64 (25.6%) to 27 (10.8%) while healthy weight, overweight and obese increased to 179 (71.6%), 26 (10.4%) and 18 (7.2%) respectively for the children fed on jollof rice with *Moringa oleifera* leaf powder. However, for the group fed on jollof rice only, there was a slight increase in under weight and healthy weight from 83 (33.2%) to 85 (34%) and 144 (57.6%) to 149 (59.6%) respectively whlile overweight and obese reduced slightly from 13 (5.2%) to 12 (4.8%) and 10 (4%) to 4 (1.6%) respectively over six months. The improvement in the nutritional status of the children who were fed on jollof rice with *Moringa oleifera* leaf powder may be due to the high concentrations of proteins, vitamins and micronutrients in *Moringa oleifera* leaf that promote healthy digestion and absorption. It may also be due to the presence of phytochemical such as phytic acid in *Moringa oleifera* leaf that increase gut transit time and result in more efficient absorption of nutrients (Muraoka and Miura, 2004). *Moringa oleifera* leaf contains both essential and non essential amino acids (Igwilo *et al*, 2011) and proteins the building blocks of the body are made from amino acids.

5.4 Effect of *Moringa oleifera* leaf powder on haematological parameters

With reference to haematological parameters, the children who were fed on jollof rice with *Moringa oleifera* leaf powder showed improvement in the haematological parameters such as packed cell volume, haemoglobin, white blood cell count, platelets, protein and iron when compared with those fed on jollof rice only.

The packed cell volume showed a significant improvement from 30% to 32% for the children who were fed on jollof rice with *Moringa oleifera* leaf powder while there was no significant difference in the group who were fed on jollof rice only from 29% to 29%. This corroborates with the work of Ugwu *et al* (2013) who observed that *Moringa oleifera* leaf extract boosted red blood cell counts in rats and has the ability to repair damages caused by merozoites to the red blood cell in mice that were infected with malaria. Ambi *et al* (2006) also observed that *Moringa*

oleifera leaf extract boosted the packed cell volume in rats. Packed cell volume is used to assess anaemia (reduced oxygen-carrying capacity), erythrocytosis, haemodilution and haemoconcentration. The purpose of red blood cells is to transfer oxygen from the lungs to body tissues. Therefore a blood sample's packed cell volume (red blood cell volume percentage) can become a point of reference of its capability of delivering oxygen. A decrease in packed cell volume indicates anaemia (Dacie and Lewis, 2000). In the bone marrow, iron is stored and used as needed to make new red blood cells. The improved packed cell volume in the children fed on jollof rice with *Moringa oleifera* leaf may be as a result of high concentration of iron in *Moringa oleifera* leaf (Gopalan *et al*, 1989).

The haemoglobin of the children who were fed on jollof rice with *Moringa oleifera* leaf powder showed a significant improvement from 10.08g/dl to 10.78g/dl while the group who were fed on only jollof rice had no significant difference from 9.92g/dl to 9.96g/dl over six months period. This finding agrees with Ugwu *et al,* (2013) who observed a significant increase in the haemoglobin of rats fed with *Moringa oleifera* leaf but is in contrast with Adegbite *et al* (2016) who observed no significant increase in the haemoglobin of people whose diet was supplemented with *Moringa oleifera* leaf. Urbain *et al* (2013) reported no significant improvement in haemoglobin among malnourished children in Bukina Faso who were given porridge with *Moringa oleifera* leaf. Haemoglobin is the protein molecule in red blood cells that carries oxygen from the lungs to the body tissues and returns carbon dioxide from the tissues back to the lungs. It is made up of four protein molecules (globulin chains) that are connected together. Haemoglobin molecule fills up the red blood cells, transports oxygen and gives the blood cell its red colour, the higher the haemoglobin concentration, the higher its ability to transport oxygen throughout the body. The improved haemoglobin may be due to the high concentration of essential and non essential amino acids in *Moringa oleifera* leaf (Igwilo *et al*, 2011), which can promote healthy circulatory system.

The white blood cell count of the children who were fed on jollof rice with *Moringa oleifera* leaf powder showed a highly significant improvement from $5.88x10^9/l$ to $6.17\ x10^9/l$ whereas a slight significant difference was observed in the group who were fed on only jollof rice from $5.68\ x10^9/l$ to $5.80\ x10^9/l$. This is consistent with the work of Ugwu *et al,* (2013) and Ambi *et al*., (2006) who observed a significant increase in total white blood cell count of rats fed with ethanol leaf extract of *Moringa oleifera* when compared to control group. White blood cells are involved

in protecting the body from infections (Saladin, 2007), and are usually raised in acute infection but may be low in overwhelming infection (Seriki *et al,* 2015). Amongst other functions, they kill virus-infected cells, enhance the production of antibodies and engulf foreign materials (antigens) that enter the body. A higher presence would therefore connote a threat to normal health. The reason for the improvement within the normal range may be attributed to the fact that *Moringa oleifera* leaf has very strong concentration of Vitamins A, C and phythochemicals like saponin and flavonoids which are antioxidants that ward off free radicals and strengthen the immune system and ability of the human body to fight infection and disease. It may also be the reason for lack of reinfection in the group that was fed on jollof rice with *Moringa oleifera* leaf powder.

The Neutrophil of the children who were fed on jollof rice with *Moringa oleifera* leaf showed a significant reduction from 43.92% to 39.35% while that of the group who were fed on only jollof rice did not show any significant difference from 46.67% to 46.52%. The finding contrasts with Adegbite *et al*, (2016) who observed a rise in the Neutrophil count of humans who were given *Moringa oleifera* leaf with pap from 2.075 to 2.245.

The lymphocyte of the children who were fed on jollof rice with *Moringa oleifera* leaf improved significantly from 48.84% to 51.25% and from 48.10% to 48.68% in children who were fed on jollof rice only. This finding contrast with Adegbite *et al*, (2016) who observed a reduction in the lymphocyte of students that were given *Moringa oleifera* leaf with pap. The increase may be because the jollof rice was prepared with red palm oil and oil increases the bioavailability of *Moringa oleifera* leaf. There are two main types of lymphocytes; B cells and T cells. The B cells produce antibodies that attack bacteria and toxins and T cells destroy bodys's own cells that have themselves been taken over by viruses or become cancerous. The improved lymphocyte may account for the reduced malaria parasitaemia on the children who were fed on jollof rice with *Moringa oleifera* leaf powder.

The monocyte of the children who were fed on jollof rice with *Moringa oleifera* leaf had a highly significant improvement from 2.17% to 3.21% while those fed on jollof rice only also increased significantly from 2.21% to 2.52%. Monocytes are a type of white blood cell and as part of the vertebrate innate immune system, influence the process of adaptive immunity. They also help other white blood cells remove damaged or dead tissues, destroy cancer cells and

regulate immunity against foreign substances. The improved monocyte may be as a result of the presence of phythochemicals like saponins and flavonoids that have antioxidant effect and reduce stress on the immune system for effective functioning (Mamadaieva *et al*, 2011). Its highly significant improvement on the group fed on jollof rice with *Moringa oleifera* leaf powder may have increased immunity and may be the reason of none reinfection over six months.

The Eosinophil of the children who were fed on jollof rice with *Moringa oleifera* leaf increased from 2.31% to 3.32% showing a significant improvement, whereas a slight significant difference was observed in the group that were fed on only jollof rice from 2.78% to 2.99%. Eosinophils are a type of white blood cells responsible for combating multicellular parasites. It also plays a role in protective immunity against parasites and contributes to inflammation that occurs in allergic disorder. The improvement may be due to high concentrations of vitamins in *Moringa oleifera* leaf that nourishes the body's immune system (Gopalan *et al,* 1989). This high improvement may be the reason for absence of malaria parasitaemia in the children who were fed on jollof rice with *Moringa oleifera* leaf powder during the follow up period.

The platelets of the children fed on jollof rice with *Moringa oleifera* leaf powder showed a significant increase from 273.17mcl to 286.83mcl while the group that were fed on only jollof rice had no significant improvement from 279.15mcl to 279.33mcl. This findings are in agreement with Adegbite *et al,* (2016) with increase from 172.5 x 10^{3} to 228 x 10^{3} among Anatomy and Physiology students of both sexes from Bingham University, Karu, Nasarawa State, Nigeria. Lowell (1989) also observed that dietary component of *Moringa Oleifera* increased platelet count in rats.

Platelets are important in the formation of platelet plugs during normal homeostasis, clot retraction and coagulation factor activation. This observation thus appears promising as a possible dietary choice for treating thrombocytopenia. The reason for the increase may be because according to Gopalan *et al* (1989), *Moringa oleifera* leaf promotes normal liver function and thrombopoietin, a glycoprotein hormone produced by the liver and kidney regulates the production of platelets. Moreover, *Moringa oleifera* leaf is full of vitamins and minerals which are necessary for healthy production of blood elements including platelets.

The protein of the children who were fed on jollof rice with *Moringa oleifera* leaf powder showed a significant improvement from 68.74g/l to 73.45g/l when compared with the group that were fed on jollof rice only that showed a reduction from 73.33g/l to 71.71g/l. This finding

agrees with Igwilo *et al* (2011) that *Moringa oleifera* leaf powder contains both essential and non-essential amino acids.and proteins, the building blocks of our bodies are made of amino acids. According to Gopalan *et al* (1989), *Moringa oleifera* leaf contains 2 times the protein found in yogurt. The reason for the improved protein on the children fed on jollof rice with *Moringa oleifera* leaf powder may be because *Moringa* leaves contain both essential and non-essential amino acids.

The iron of the children who were fed on jollof rice with *Moringa oleifera* leaf powder improved significantly from 65.29µg/dl to 77.30 µg/dl but there was no significant difference in the iron of the children fed on jollof rice only from 74.63 µg/dl to 74.38 µg/dl. This significant increase of iron in the children fed on jollof rice with *Moringa oleifera* leaf powder may be because of the strong concentration of iron in *Moringa oleifera* leaf. According to Gopalan *et al* (1989), *Moringa oleifera* leaf has 0.75 times the iron found in spinach. Iron plays an important role in the synthesis of haemoglobin in blood which carries oxygen to all parts of the body. It may also be because according to Anwar *et al*, (2007), *Moringa oleifera* leaf is rich in vitamin C which increases iron absorption in the body.

5.5 Conclusion

The study showed good acceptability of jollof rice with *Moringa oleifera* leaf powder among the children as none of them had any reaction during the feeding period. The results of this study supported other reports about *Moringa oleifera* leave extract having medicinal effect that can effectively protect animals against *Plasmodium* by preventing the establishment of new infections and progression of the disease into heavy attacks (Obasi and Mba 2010).

It could also be beneficial to red blood cell formation in humans as seen in the improvement of haematological parameters among children fed on jollof rice with *Moringa oleifera* leaf powder.

It could therefore be concluded that *Moringa oleifera* leaf is highly nutritious and could be used as a dietary supplement and may contribute to the fight against malnutrition in Nigeria based on its nutrient content as reported by other researchers.

This is because its haematopoietic activity could boost immunity; reduce the frequency of malaria parasitaemia among children and thereby reduce the problem of drug resistance in malaria because people who do not have malaria are less likely to abuse antimalaria drugs.

5.6 Recommendations

Moringa oleifera leaf powder is recommended for inclusion in the package of nutritional interventions for the improvement of nutritional status of children less than five years for reducing the global burden of malaria disease.

Moringa oleifera leaf powder can also be introduced into the school feeding programme in Nigeria as already been done in India since 2002. Since there is lack of adequate information by parents and caregivers about appropriate feeding practices and essential types and varieties of foods that children require for growing up healthy, there should be massive health education to mothers and care givers on the nutritional potentials of *Moringa oleifera* leaf and its way of preparation to salvage the nutrients.

In view of the favourable amino acid profile of *Moringa oleifera* leaves and their wide and ready availability throughout the tropics and subtropics especially Nigeria, it can be considered as a potential feed component with high nutritive value for school children. Since the technology for cultivating *Moringa* is cheap and affordable both in rural and urban areas in Nigeria, more people could be encouraged to start processing and packaging *Moringa oleifera* leaf powder as an income generating activity so as to alleviate poverty among community members in Nigeria.

5.7 Contribution to Knowledge

The study showed that *Moringa oleifera* leaf which is a local vegetable available in Nigeria both during the dry and wet season boosted immunity in children and reduced the frequency of malaria episodes in chidren under five years.

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Appendix 4: Informed Consent Form

My name is Ogolo Bernice Amala a Principal Nursing Officer and PhD student of Public Health Parasitology in the Department of Parasitology and Entomology, Nnamdi Azikiwe University Awka. I am doing a research on the antimalaria effect of *Moringa oleifera* leaf powder on the nutritional status and malaria re-infection rate of nursery school children aged 2-5 years in Oba Idemili South L.G.A Anambra State. The test involves withdrawing 4mls of venous blood with a sterile needle from your child's arm by a phlebotomist (research assistant) to be paid by the researcher. The blood will be used to test for malaria parasite and hematological values. Those positive for malaria parasite will be given an antimalarial drug (coartem) by the researcher and their blood rechecked for parasite clearance after three weeks. Half of the children will be fed with jollof rice supplemented with 8g of *Moringa oleifera* leaf powder while the other half will be fed with jollof rice without *Moringa oleifera* leaf powder (control) as a daily lunch pack for six months.

Benefit of the study to the participants

1. Free laboratory test for malaria parasite, hematological parameters and anthropometry
2. Antimalaria treatment with coartem for those positive for malaria
3. Free lunch to be provided by the researcher for six months

Benefit of the study to the society

- *Moringa oleifera* leaf powder a cheap and affordable local resource can be used to reduce the frequency of malaria infection and drug resistance in malaria.
- *Moringa oleifera* leaf powder can be added to the diet of children as part of the school feeding program to improve nutritional deficiencies and promote physical and mental development since the technology for cultivating *moringa* is cheap and affordable both in rural and urban areas in Nigeria (instead of relying on external funding for vitamin A supplement).
- *Moringa oleifera* leaves could be promoted in the National Feeding Programme to enhance the vitamin A status of the populations.

- I solicit your support in this project by allowing your child to be one of the participants.
- You are assured that no harm will come to your child as a result of the study.

- Your child's participation in this study is voluntary.
- You are free to withdraw your child from the study any time you wish.
- Finally, you are assured that any medical information you give or we find out during the course of this study will be strictly confidential.
- You are free to ask for further explanations at any time.
- There will be no financial involvement on the participants and no financial benefits too.
- In case of any problem regarding this study, please contact the following phone number: 07030196887. Thank you.

Please sign below if you wish your child to be part of this study.

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Name and Signature of Parent/Guardian	Name and Signature of Researcher
Date…………………………….	Date…………………………….
	Phone number of Researcher 07030196887

Appendix 5: Data Analysis

Objective 1: The prevalence and intensity of malaria infection among children 4 to 5 years in Oba community.

Malaria Prevalence among children aged 4 – 5 in Oba Community

	MP Positivity		Total
	Negative	Positive	
Number	429	71	500
Percentage	85.8	14.2	100

SEX		MP Positivity		Total Number Examined
		Negative	No infected	
Male	Number of Males	188	29	217
	%	86.6%	13.4%	100.0%
Female	Number of Females	241	42	283
	%	85.2%	14.8%	100.0%
Total	Total Number	429	71	500
	% within Sex	85.8%	14.2%	100.0%

Thus the prevalence rate is about 14.2%

Malaria Intensity among children aged 4 – 5 in Oba Community

Variables	Values
Sum	388
Number Positive	71
Mean	5.4648
Standard Deviation	2.8377

Thus the intensity of malaria in the area is about 5.4648 $\pm$ 2.8377

Objective 2: The frequency of malaria parasite reinfection among children fed with *Moringa oleifera* leaf powder and children not fed with *Moringa oleifera* leaf powder.

Null hypothesis: *Moringa oleifera* leaf has no effect on the frequency of malaria reinfection in children

Alternative hypothesis: *Moringa oleifera* leaf has an effect on the frequency of malaria reinfection in children.

Frequency of Malaria parasite infection recorded in the various replicates.

Replicates	Negative (%)	Positive (%)	Total
First Rep	429 (85.8)	71 (14.2)	500
Second Rep	454 (90.8)	46 (9.2)	500
Third Rep	500 (100)	0 (0)	500
Fourth Rep	464 (92.8)	36 (7.2)	500
Total	1847 (92.4)	153 (7.6)	2000

$\chi^2 = 73.626$, P-Value = 0.00

Since $p<0.05$, we reject the null hypothesis and conclude that *Moringa oleifera* leaf has an effect on the frequency of malaria in children.

Objective 3: Effect of *Moringa oleifera* leaf powder on the nutritional status of children.

Null Hypothesis: *Moringa oleifera* leaf cannot improve the nutritional status in children

Alternative Hypothesis: *Moringa oleifera* leaf can improve the nutritional status in children

	Extract	N	Mean	Std. Deviation	t-test	p-value	Remark
Weight	Moringa	1000	20.7367	2.51642	20.140	0.000	Sig
	Placebo	1000	18.3945	2.68192			
Height	Moringa	1000	120.0458	59.82337	5.508	0.000	Sig
	Placebo	1000	109.5730	6.07450			
MUAC	Moringa	1000	16.2529	1.24486			Sig
	Placebo	1000	15.5253	.94516	14.721	0.000	
	Placebo	1000	74.4900	12.92730			

Since $p<0.05$, we reject the null hypothesis and conclude that *Moringa oleifera* leaf can improve the nutritional status in children.

Effect of *Moringa oleifera* leaf powder on the Weight of children

Extract	Replicates	Mean	Std. Deviation	N	F-value	P-value	Remark
Moringa	1.00	20.1155	2.38969	250	7.696	0.000	Sig
	2.00	20.7820	2.45766	250			
	3.00	20.9072	2.48322	250			
	4.00	21.1416	2.62356	250			
	Total	20.7360	2.51527	1000			
Placebo	1.00	18.5000	2.67922	250	0.257	0.856	Not Sig
	2.00	18.3320	2.71959	250			
	3.00	18.3220	2.69376	250			
	4.00	18.4180	2.65035	250			
	Total	18.3929	2.68278	1000			

Effect of *Moringa oleifera* leaf powder on the Height of children

Extract	Replicates	Mean	Std. Deviation	N	F-value	P-value	Remark
Moringa	1.00	116.4900	5.72948	250	0.298	0.827	Not. Sig
	2.00	116.8160	5.83789	250			
	3.00	116.8604	5.75384	250			
	4.00	116.9454	5.80207	250			
	Total	116.7775	5.77480	1000			
Placebo	1.00	109.5542	6.05270	250	0.012	0.298	Not Sig
	2.00	109.5920	6.10168	250			
	3.00	109.5560	6.09026	250			
	4.00	109.6426	6.14206	250			
	Total	109.5863	6.08779	1000			

Effect of *Moringa oleifera* leaf powder on the MUAC of children

Extract	Replicates	Mean	Std. Deviation	N	F-value	P-value	Remark
Moringa	1.00	15.7203	1.10292	250	24.082	0.000	Sig
	2.00	16.3136	1.14883	250			
	3.00	16.3820	1.09480	250			
	4.00	16.5912	1.43850	250			
	Total	16.2509	1.24527	1000			
Placebo	1.00	15.4056	.87876	250	2.05	0.105	Sig
	2.00	15.5464	.96339	250			
	3.00	15.5496	.95351	250			
	4.00	15.6068	.97947	250			
	Total	15.5273	.94616	1000			

Objective 4: Effect of *Moringa oleifera* leaf powder on the hematological parameters such as packed cell volume, white blood cell count, hemoglobin, iron and protein of children

Null Hypothesis: *Moringa oleifera* leaf cannot boost immunity by improving the hematological parameters in children

Alternative Hypothesis: *Moringa oleifera* leaf can boost immunity by improving the hematological parameters in children

	Extract	N	Mean	Std. Deviation	t-test	p-value	Remark
PCV	Moringa	1000	.3130	.02378	15.865	0.000	Sig
	Placebo	1000	.2976	.01929			
HB	Moringa	1000	10.4325	.79270	15.868	0.000	Sig
	Placebo	1000	9.9202	.64312			
WBC	Moringa	1000	6.0296	.99441	7.601	0.000	Sig
	Placebo	1000	5.7176	.83435			
Neutrophil	Moringa	1000	43.5333	9.96693	-8.499	0.000	Sig
	Placebo	1000	46.7540	6.65355			
lymphocyte	Moringa	1000	49.8800	7.87258	5.149	0.000	Sig
	Placebo	1000	48.0100	8.36085			
Monocyte	Moringa	1000	2.7530	1.33708	8.263	0.0000	Sig
	Placebo	1000	2.3110	1.03603			
Eosinophil	Moringa	1000	2.9360	1.44083	1.681	0.093	Sig
	Placebo	1000	2.8410	1.05680			
Platelets	Moringa	1000	281.7455	70.73636	1.012	0.312	Sig
	Placebo	1000	278.6020	68.14941			
Protein	Moringa	1000	72.0310	8.41266	-3.226	0.001	Sig
	Placebo	1000	73.1490	7.02126			
Iron	Moringa	1000	72.3220	18.33637	-3.056	0.002	Sig
	Placebo	1000	74.4900	12.92730			

Since $p<0.05$, we reject the null hypothesis and conclude that *Moringa oleifera* leaf can boost immunity by improving the hematological parameters in children

Effect of *Moringa oleifera* leaf powder on the PVC of children

Extract	Replicates	Mean	Std. Deviation	N	F-value	P-value	Remark
Moringa	1.00	.3025	.02706	250	36.497	0.000	Sig
	2.00	.3117	.02088	250			
	3.00	.3141	.02060	250			
	4.00	.3235	.02105	250			
	Total	.3129	.02374	1000			
Placebo	1.00	.2977	.01912	250	0.830	0.478	Sig
	2.00	.2963	.02117	250			
	3.00	.2975	.01906	250			
	4.00	.2990	.01800	250			
	Total	.2976	.01937	1000			

Effect of *Moringa oleifera* leaf powder on the HB of children

Extract	Replicates	Mean	Std. Deviation	N	F-value	P-value	Remark
Moringa	1.00	10.0837	.90198	250	36.539	0.000	Sig
	2.00	10.3908	.69592	250			
	3.00	10.4704	.68683	250			
	4.00	10.7837	.70177	250			
	Total	10.4315	.79135	1000			
Placebo	1.00	9.9222	.63747	250	0.832	0.477	Sig
	2.00	9.8773	.70557	250			
	3.00	9.9172	.63574	250			
	4.00	9.9682	.59979	250			
	Total	9.9212	.64558	1000			

Effect of *Moringa oleifera* leaf powder on the WBC of children

Extract	Replicates	Mean	Std. Deviation	N	F-value	P-value	Remark
Moringa	1.00	5.8846	1.05582	250	3.793	0.010	Sig
	2.00	5.9912	1.03900	250			
	3.00	6.0672	.98395	250			
	4.00	6.1739	.87603	250			
	Total	6.0289	.99552	1000			
Placebo	1.00	5.6811	.82555	250	1.301	0.273	Sig
	2.00	5.6812	.82570	250			
	3.00	5.7036	.82225	250			
	4.00	5.8068	.85753	250			
	Total	5.7183	.83329	1000			

Effect of *Moringa oleifera* leaf powder on the Neutrophil of children

Extract	Replicates	Mean	Std. Deviation	N	F-value	P-value	Remark
Moringa	1.00	43.9203	6.24705	250	23.302	0.000	Sig
	2.00	45.0880	6.10420	250			
	3.00	45.9200	6.37465	250			
	4.00	39.3550	15.83609	250			
	Total	43.5754	9.89221	1000			
Placebo	1.00	46.6707	6.94128	250	0.118	0.950	Not Sig
	2.00	46.8360	6.50124	250			
	3.00	46.8200	6.55373	250			
	4.00	46.5215	7.15289	250			
	Total	46.7119	6.78391	1000			

Effect of *Moringa oleifera* leaf powder on the lymphocyte of children

Extract	Replicates	Mean	Std. Deviation	N	F-value	P-value	Remark
Moringa	1.00	48.8406	6.31494	250	4.387	0.004	Sig
	2.00	50.0400	6.33838	250			
	3.00	49.4000	11.12904	250			
	4.00	51.2570	6.44066	250			
	Total	49.8820	7.87211	1000			
Placebo	1.00	48.1084	7.30391	250	1.792	0.147	Sig
	2.00	48.2200	7.30046	250			
	3.00	47.0160	10.69435	250			
	4.00	48.6853	7.61055	250			
	Total	48.0080	8.36085	1000			

Effect of *Moringa oleifera* leaf powder on the Monocyte of children

Extract	Replicates	Mean	Std. Deviation	N	F-value	P-value	Remark
Moringa	1.00	2.1753	1.22684	250	30.449	0.000	Sig
	2.00	2.6640	1.48861	250			
	3.00	2.9600	1.20840	250			
	4.00	3.2169	1.17825	250			
	Total	2.7530	1.33708	1000			
Placebo	1.00	2.1807	1.07176	250	5.430	0.001	Sig
	2.00	2.2360	1.09961	250			
	3.00	2.3000	1.04228	250			
	4.00	2.5259	.89125	250			
	Total	2.3110	1.03603	1000			

Effect of *Moringa oleifera* leaf powder on the Eosinophil of children

Extract	Replicates	Mean	Std. Deviation	N	F-value	P-value	Remark
Moringa	1.00	2.3108	1.31112	250	25.663	0.000	Sig
	2.00	2.9440	1.49325	250			
	3.00	3.1640	1.43154	250			
	4.00	3.3253	1.31776	250			
	Total	2.9350	1.44113	1000			
Placebo	1.00	2.7831	1.15753	250	2.405	0.066	Sig
	2.00	2.7840	1.09459	250			
	3.00	2.8040	1.01672	250			
	4.00	2.9960	.93594	250			
	Total	2.8420	1.05648	1000			

Effect of *Moringa oleifera* leaf powder on the Platelets of children

Extract	Replicates	Mean	Std. Deviation	N	F-value	P-value	Remark
Moringa	1.00	273.1793	65.20371	250	3.070	0.027	Sig
	2.00	277.4840	65.65507	250			
	3.00	289.8760	72.60304	250			
	4.00	286.8333	78.20473	250			
	Total	281.8295	70.82690	1000			
Placebo	1.00	279.1566	66.72406	250	0.039	0.990	Not Sig
	2.00	277.6120	68.98121	250			
	3.00	277.9680	68.89500	250			
	4.00	279.3347	67.96923	250			
	Total	278.5180	68.05132	1000			

Effect of *Moringa oleifera* leaf powder on the Protein of children

Extract	Replicates	Mean	Std. Deviation	N	F-value	P-value	Remark
Moringa	1.00	68.7410	8.46408	250	26.382	0.000	Sig
	2.00	71.2000	8.15512	250			
	3.00	74.7240	7.80844	250			
	4.00	73.4578	8.00852	250			
	Total	72.0260	8.41654	1000			
Placebo	1.00	73.3373	6.89193	250	0.185	0.907	Not Sig
	2.00	73.1240	6.96842	250			
	3.00	73.2560	7.06130	250			
	4.00	72.9004	7.17175	250			
	Total	73.1540	7.01580	1000			

Effect of *Moringa oleifera* leaf powder on the Iron of children

Extract	Replicates	Mean	Std. Deviation	N	F-value	P-value	Remark
Moringa	1.00	65.2908	19.13487	250	22.977	0.000	Sig
	2.00	71.1280	17.71165	250			
	3.00	75.7000	17.84724	250			
	4.00	77.3012	16.30090	250			
	Total	72.3430	18.35719	1000			
Placebo	1.00	74.6386	13.07415	250	0.030	0.993	Not Sig
	2.00	74.5280	13.20617	250			
	3.00	74.3240	13.40065	250			
	4.00	74.3865	11.95450	250			
	Total	74.4690	12.90122	1000			