PREVALENCE OF MALARIA AMONG PREGNANT WOMEN ATTENDING ANTENATAL CLINIC AND KNOWLEDGE AND ATTITUDE OF CAREGIVERS TO MALARIA DIAGNOSIS IN OZUBULU, ANAMBRA STATE.

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TITLE PAGE

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A PROJECT SUBMITTED BY

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CERTIFICATION

This is to certify that the project Prevalence of Malaria among Pregnant Women Attending Antenatal Clinic and Knowledge and Attitude of Caregivers to Malaria Diagnosis in Ozubulu, Anambra State was approved for Obianumba, Stella Nnenna, NAU/M. Sc/2009536015F as a Master's Thesis in the Department of Parasitology and Entomology, Faculty of Biosciences, Nnamdi Azikiwe University, Awka, Anambra State.

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DEDICATION

Dedicated to Almighty God, for His Grace.

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May God bless and reward you all in Jesus name, Amen!

ABSTRACT

A study was conducted to determine the prevalence of malaria parasite infection among pregnant women using peripheral blood and microscopy in Ozubulu. Also the knowledge and attitude of caregivers to malaria diagnosis were determined. The study was carried out between November 2011 and January 2012. A total of 243 pregnant women were sampled for malaria parasites infection using thick and thin film smears and 393 caregivers were sampled for knowledge and attitude in malaria diagnosis. The result showed a prevalence of 53.9% (131/243). It was found that women in their first pregnancy had the highest prevalence of 65.8% (48/73). Highest prevalence of 71.6% (58/81) was also recorded among women in their first trimester. Pregnant women aged 21 -25 years had highest infection rate of 68.8% (33/48). The result also showed that all the caregivers interviewed knew about self diagnosis of malaria. 57% (224/393) of the caregivers knew about microscopy, while only 2.5% (10/393) knew about Rapid Diagnostic Tests. 40.2% (158/393) of the caregivers rated laboratory diagnosis as not important, 47.8% (188/393) as barely important and 12% (47/393) as very important. 38.7% (152/393) of the caregivers suggested use of community health workers in enlightenment program on malaria diagnosis. Following the high prevalence of malaria infection in pregnancy, more efforts are needed in the control of malaria in pregnancy. The people need public enlightenment in the importance of malaria diagnosis.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE

INTRODUCTION

Malaria is a life threatening parasitic disease transmitted by female *Anopheles* mosquitoes. More than 40% of the world population lives in malarious areas (WHO, 2010). It is estimated that the number of cases of malaria rose from 233 million in 2000 to 244 million in 2005 but decreased to 225 million in 2009 (WHO, 2010). The number of deaths due to malaria is estimated to have decreased from 985 000 in 2000 to 781 000 in 2009 (WHO, 2010). Malaria is the most highly prevalent tropical disease with high morbidity and mortality, and with high economic and social impact (WHO, 2010). Over 90% of all deaths caused by malaria occur in sub - Saharan Africa and about 85% of deaths globally were in children under 5 years of age (WHO, 2010). In addition, pregnant women are at immense risk of malaria due to natural immune depression in pregnancy (Fievet *et al.,* 2007). About 25% of all estimated malaria cases in the World Health Organisation African Region occur in Nigeria (WHO, 2010).

Malaria infection during pregnancy is a major public health problem in tropical and subtropical regions throughout the world (WHO, 2010). The burden of malaria infection during pregnancy is caused mainly by *Plasmodium falciparum*, the most common malaria species in Africa (WHO, 2010). Each year at least 3 million pregnancies occur among women in malarious areas of Africa, most of who reside in areas of relatively stable malaria transmission

(Brabin, 2000). The symptoms and complications of malaria during pregnancy differ with the intensity of malaria transmission and thus with the level of immunity the pregnant woman has acquired (Perlmann and Troye-Blomberg (2000). Pregnant women and the unborn children are particularly vulnerable to malaria, which is a major cause of prenatal mortality, low birth weight, and maternal anaemia (Greenwood *et al*., 2007).

Beyond the impact of malaria on children and pregnant women, it affects the general population. 100% of the total population of Nigeria is at risk of malaria and at least 50% of the total population suffers from at least one episode of malaria each year (WHO, 2010). About 51% of malaria cases and deaths in Nigeria occur in rural villages away from effective diagnostic or treatment facilities (WHO, 2010). Malaria cases and deaths have been increasing in the country, mainly due to injudicious use of antimalarial drugs, delayed health seeking, and reliance on the clinical judgment without laboratory confirmation in most of the peripheral health facilities (Vander *et al*., 2005). Despite evidence of the cost-effectiveness of improving treatment access and compliance (Goodman *et al.,* 1999), most victims of malaria still die because of a lack of health care close to their homes or because their condition is not diagnosed by health workers (WHO, 2000; Armstrong-Schellenberg *et al*., 1994). Early diagnosis and prompt effective treatment of malaria illness has been a cornerstone of malaria control (Vander *et al.,* 2005). Diagnosis based on symptoms alone has inherent difficulties (Vander *et al*., 2005), although volunteer

health workers in rural areas have practised it with some success (Pagnoni, 1997; Okanurak and Ruebush, 1996). The reduction of morbidity and the interruption of parasite transmission by means of community-based antimalarial treatment require an accurate, rapid and practical method of diagnosis. The delivery of treatment in rural areas in Nigeria is complicated by the centralized nature of microscopy services (Alaba and Alaba, 2008). Over the past few years, developments in rapid field diagnostic techniques based on the demonstration of parasite antigens have opened new possibilities for improved rural malaria diagnosis that is independent of centralized diagnostic services (Bojang, 1999; Singh *et al*., 1997).

There have been a considerable number of reports about knowledge, attitudes, and practices relating to malaria and its control from different parts of Africa. These reports concluded that misconceptions concerning malaria still exist and that practices for the control of malaria have been unsatisfactory (Deressa *et al.,* 2008). However, epidemiological patterns of malaria are widely different from one place to another (Himeiden *et al*., 2005). Specific data of a place collected can help in the making of a design of improved programme for strategic malaria control for a particular location. There are available effective low-cost strategies for the treatment of malaria, but any attempt to control a disease such as malaria in an area or locality should first of all be preceded by an extensive evaluation of the magnitude of the prevailing situation; a complete description of the health problems of the community

comprising an account not only of the prevalence, but also of the community's view of its own problems and its use of existing health services. Ascertaining the factors that influence community and provider acceptance of and adherence to the new treatment regime will be vital to improving the effectiveness of this intervention and reducing the risk of development of drug resistance, and thus reduction in prevalence, towards elimination and subsequent eradication. Part of the rationale for investigating malaria prevalence in pregnancy is to compare present with past situations especially with current efforts at controlling malaria during pregnancy.

The aim of this study was to determine the prevalence of malaria infection among pregnant women attending antenatal clinic, as well as the knowledge and attitude of caregivers to malaria diagnosis in Ozubulu, Anambra State, Southeast Nigeria. The specific objectives include:

i. To determine the prevalence of malaria infection among pregnant women attending antenatal clinics in Ozubulu, Anambra State, Southeast Nigeria.
ii. To determine the knowledge and attitude of caregivers to malaria diagnosis in the rural community.

CHAPTER TWO

LITERATURE REVIEW

2.1 Trends in Prevalence of Malaria in Pregnancy

Plasmodium falciparum infection is the major cause of morbidity and mortality particularly among the vulnerable groups (Warsame *et al.,* 2005). Pregnant women constitute the main adult risk group for malaria. In Nigeria, the National Malaria Control Programme (NMCP) reported 4.3 million suspected malaria cases in 2009; 42% increase compared to 2000 (WHO, 2010).

In areas of high *P. falciparum* transmission in Africa, anaemia is the most common form of severe malaria (Aribodor *et al.,* 2003) and there is a seasonal drop in haemoglobin concentration in children during the high transmission season, probably due to increased malaria transmission (Diadier *et al.,* 2007). Generally, there is slow acquisition of active immunity to malaria (Perlmann and Troye-Blomberg, 2000). The very low prevalence in young infants is consistent with maternal immunity, but the possibility that social practices reduce the exposure of very young infants to mosquitoes cannot be excluded (Plebanski and Hill, 2000). Children born to immune mothers are protected against the disease during their first half year of life by maternal antibodies. As they grow older, after continued exposure from multiple infections with malaria parasites over time, they build up an acquired immunity and become relatively protected against disease and blood stage parasites (Plebanski and Hill, 2000),

hence lower prevalence of malaria among the older age groups (Olasehinde, 2010). The age distribution of parasite prevalence and parasitaemia density provide suggestive information about the level of naturally acquired immunity to malaria and, indirectly, about the long term intensity and stability of malaria transmission (Perlmann and Troye-Blomberg, 2000).

Malaria in pregnancy is a significant health problem in sub-Saharan Africa where 90% of the global malaria burden occurs. Malaria disease is more hazardous especially an infection with *P. falciparum* during pregnancy. *P. falciparum* malaria can run a turbulent and dramatic course in pregnant women. Pregnancy appears to interfere with the immune processes in malaria, a disease which itself alters immune reactivity (Perlmann and Troye-Blomberg, 2000). The physiological changes of pregnancy and the pathological changes due to malaria have synergistic effect on each other, thus making life difficult for both the mother and the child (Steketee *et al.,* 2001). In pregnancy, malaria tends to be more atypical in presentation. This could be due to the hormonal, immunological and haematological changes during pregnancy (Plebanski and Hill, 2000). In highly endemic malarious area such as Nigeria, where semi-immune adults usually have substantially acquired resistance to local strains of *Plasmodia*, the prevalence of clinical malaria is higher and its severity greater in pregnant women than non-pregnant women (Uko *et al.,* 1998). Pregnant women with *falciparum* malaria are significantly more anaemic than non-infected pregnant women or infected non-pregnant women (Mockenhaupt *et al.,* 2000). At pregnancy, immunity has been altered; hence, with malaria 70-

80% of pregnant women in malarious areas are susceptible to anaemia (Brabin, 1996). *Falciparum* infection is higher during pregnancy, more so in primigravidae and is usually associated with anaemia or reduced haemoglobin levels (Mockenhaupt *et al*; 2000). Anaemia is the trademark of malaria, especially with *P. falciparum* infection. The mean haematocrit level is lower in primigravidae when compared with secundigravidae and multigravidae in malaria endemic areas (Nosten *et al.,* 1991). Cell-mediated immune responses to malaria antigens are more markedly suppressed in first than in subsequent pregnancies (Brabin, 1996). The multigravidae are presumably less affected because immunological memory from first pregnancy is retained (Brabin, 1996). Younger maternal age is also an independent risk factor for malaria in pregnancy (Espinoza *et al.,* 2005). Young primigravidae and multigravidae are at greater risk of malaria and its adverse effects than older primigravidae and multigravidae respectively because of continuous development of malaria immunity in older women. (Dicko *et al*., 2003). Human Immunodeficiency Virus (HIV) infection increases susceptibility to malaria, resulting in more prevalent and higher infection, and a relative loss of gravid-dependent immunity (Cohen *et al.,* 2005). Cerebral malaria was a common complication of severe *P. falciparum* infection, with a high mortality rate during pregnancy (Brabin, 2000). Akinboro, 2010, recorded 88% predominance of *P. falciparum* with highest prevalence (59.4%) in first trimester irrespective of parity status. *P. falciparum* infection during pregnancy increases the likelihood of maternal anaemia, abortion, still birth, prematurity, intrauterine growth retardation

and low birth weight (Mockenhaupt *et al*., 2000). Uko *et al.,* 1998 documented about 3% abortions, 3.7% stillbirths and 2.2% neonatal deaths in *P. falciparum* infected women. The presence of malaria parasites in the blood of newborns may be as a result of congenital malaria as reported by Oduwole *et al*., 2011. Congenital malaria, defined as the presence of malaria parasites in the erythrocytes of newborns aged less than 7 days, was considered rare in endemic areas until recent studies started reporting high prevalence rates (Oduwole *et al*., 2011). Though has been documented for many years, it was previously thought to be uncommon especially in indigenous populations; more recent studies, however, suggest that incidence has increased, and values between 0.30 to 33.00% have been observed from both endemic and non-endemic areas (Obiajunwa *et al*., 2005). The prevalence of *P. falciparum* infection is higher in the wet season than in the dry season (Ayanda, 2009). Minakaw *et al*., 2002 opined that the rainy season presents favourable environmental conditions that enhance mosquito breeding and survival, through the proliferation of larval habitats and improved humidity respectively.

2.2 Epidemiological Factors of Malaria

The burden of malaria varies across different regions of the world and even within a country (O'Meara *et al.,* 2009). This is driven by the variation in parasite–vector– human transmission dynamics that favour or limit the transmission of malaria infection and the associated risk of disease and death (Morse, 1995).

Human malaria is transmitted by female mosquitoes of the genus *Anopheles* from human to human. Approximately 422 species of *Anopheles* are known, of which 68 are indicated as vectors of malaria (Service, 1963). *Anopheles arabiensis* and *Anopheles gambiae* belong to the most effective vectors of malaria parasites in Africa (Coetzee, 2004). Major *Anopheles* mosquitoes in Nigeria are *An. gambiae, An. arabiensis, An. funestus* and *An. melas* (WHO, 2010). *An. gambiae s.s* is omnipresent in Nigeria, because of its indiscriminate breeding habitats (Ayanda, 2009). It is highly endophilic, anthropophagous, considered as wet season vector, but can occasionally be zoophilic and exophilic (Ayanda, 2009). In the past, most research had focused on the members of *An. gambiae* complex, but according to Ayanda, 2009 the *An. funestus* group may be as complex and problematic as the *An. gambiae* group with different biology and vectorial capacity. In some areas of Nigeria, it has been projected that it could replace *An. gambiae s.s* as the major vector of endemic malaria. Puzzling shifts in species composition of *An. arabiensis* and *An. gambiae s.s* have been observed in Nigeria (Onyabe and Conn, 2001). Githeko *et al.,* 2008 reported that *An. arabiensis* could be anthropophagous, where there are less animal hosts, as observed by Onyabe and Conn, 2001 in the Savannah - forest, where *An. arabiensis* was responsible for 34.1% of human blood meals. *An. arabiensis* appears to be a good vector of malaria, especially in the Savannah-forest (Onyabe and Conn, 2001).

Malaria vectorial system in Nigeria is more complex than expected, looking at the combined contribution of these mosquito species to malaria transmission. It is therefore very important to

understand the dynamics of the transmission of malaria in a large country like Nigeria with different ecological zones through a regular assessment of each country's malaria situation which is worthwhile since control measures can only be effective if the abundance, behaviour and proportion of the species are known.

Incidence of malaria varies by weather, which affects the ability of the main carrier of malaria parasites, *Anopheles* mosquitoes, to survive or otherwise (Mwangagia *et al.,* 2007). Tropical areas including Nigeria have the best combination of adequate rainfall, temperature and humidity allowing for breeding and survival of anopheline mosquitoes (Okwa *et al.,* 2009; Onyabe and Conn, 2001). Malaria transmission in Nigeria takes place all year round in the south but is more seasonal in the northern regions (WHO, 2010). Malaria transmission, based on climatic parameters occurred between April and October in Anambra state (Ayanlade *et al*., 2010), which shows that rainfall plays an important role in the distribution of breeding sites for the mosquito vector thereby influencing malaria transmission (Okwa *et al.,* 2009). In Nigeria, the peak malaria transmission coincides with the appearance of stagnant water collections just after the rainy season (Okwa *et al.,* 2009).

Although malaria is one of the most climate-sensitive vector-borne diseases (Morse, 1995), several other factors have been identified as contributing to its emergence and spread. These include environmental and socio-economic changes, deterioration of health care and food production systems, and the modification of microbial/vector adaptation (McMichael *et al*., 1998; Morse,

1995). In malaria endemic areas, factors such as poverty, poor socioeconomic status, poor education, lack of enlightenment and poor environmental sanitation have been attributed to availability of mosquito-friendly environment- conditions which allow for survival and proliferation of the vector and pathogenic parasite (McMichael *et al*., 1998).

Increase in population density led to an increase in human exposure and more pressure on limited productive land (Lindsay and Martens, 1998). Pressures on productive land, force farmers to clear forests and reclaim swamps. Puddles and elevated temperatures result from lost tree and ground cover, providing ideal breeding sites for mosquitoes (Walsh *et al*., 1993). According to Warsame *et al*., (1995), increased flooding could facilitate the breeding of malaria carriers in formerly arid areas. Small geographical changes in the distribution of malaria may expose large numbers of people to infection (Warsame *et al*., 1995).

2.3 Effects of Maternal Malaria on Infants

Falciparum malaria during pregnancy has long been recognized as an important determinant of low birth weight of newborns (Brabin, 2000; Menendez *et al.*, 2000). Low birth weight (LBW) which is defined as the birth weight of less than 2.5 kg is usually more marked in primigravidae (Brabin, 2000) but can extend to second and third gravidae in areas of low malaria transmission (Nosten *et al.,* 1991). In most studies designed to investigate the relation between malaria during pregnancy and birth weight, potential confounding factors, such as socioeconomic status, maternal nutrition, and smoking, have not been taken into

account (Menendez *et al.,* 2000). However, a number of randomized controlled trials of preventive antimalarial measures during pregnancy have confirmed this causal effect by showing that preventing malaria increases birth weight (Aribodor *et al.,* 2009; Menendez *et al*., 2000).

The major adverse effect of malaria in pregnancy on the mother is anaemia. Anaemia during pregnancy is a global problem, and in malaria endemic areas it is usually most severe in the second trimester of gestation, following a period of acute malaria infection in the first trimester (Brabin, 2000). Severe anaemia in pregnancy is an important contributor to maternal and pre-natal morbidity and mortality (Dicko *et al*., 2003), low-birth weight, iron and foliate deficiency, especially in first pregnancies (Mockenhaupt *et al*., 2000). Malaria during pregnancy has not been associated directly with an increase in infant mortality, whereas severe maternal anaemia has been associated with an increased risk of infant death in the prenatal (Kagu *et al.,* 2007) and post neonatal periods (Dolan *et al.,* 1993; Nosten *et al.,* 1991). However, as low birth weight is a major determinant of infant mortality (Ashworth, 1998), it has been assumed that malaria and anaemia during pregnancy would increase infant mortality indirectly by lowering birth weight. In malarious areas, though malaria and anaemia are likely to act together to reduce birth weight, their independent effects are difficult to distinguish. In a study conducted in a highly malarious area of Papua New Guinea, severe maternal anaemia was associated with low birth weight in primigravidae, whereas there was no obvious consistent association between parasite positivity and low birth weight

(Brabin, 2000). However, a more recent study conducted in Nigeria, which attempted to quantitate the separate effects of anaemia- and malaria-attributable low birth weight, concluded that in malarious areas, malaria was a more important risk factor for low birth weight than was anaemia (Aribodor *et al.*, 2009). Until recently, the distinction between full-term and preterm low birth weight was difficult in the tropics. As a consequence, the relative contributions of malaria-associated intrauterine growth retardation and preterm delivery were not clearly established. Since the introduction of accurate methods for the estimation of gestational age, it has been suggested that the relative importance of these causes of low birth weight may depend on the level of malaria transmission and the timing of malaria infection during pregnancy (Luxemburger *et al*., 2001).

Premature birth results commonly from symptomatic malaria and is usual in severe malaria. It is therefore common in low-transmission areas, where acquired premunition is poor, and in epidemics (Menon, 1972). However, in prospective studies conducted in a low-malaria-transmission setting in Thailand, infection with malaria (which was most often asymptomatic) was associated with low birth weight, resulting mainly from intrauterine growth retardation rather than preterm delivery (Dolan *et al.,* 1993). In sub-Saharan Africa where malarial transmission is generally much higher and maternal malaria is rarely associated with symptoms, some studies have demonstrated that there were different consequences on the newborn infant, depending on the timing of infection (Brabin, 2000). Parasitaemia in the antenatal period was associated with

intrauterine growth retardation, whereas cord blood parasitaemia, probably reflecting a recent active infection, was associated with premature birth (Sullivan *et al.,* 1999). In an area of much higher rates of transmission, chronic placental infection was associated with both mechanisms, and low birth weight resulting from premature birth was more common than usually thought (Menendez *et al.,* 2000).

2.4 Immune Response to Malaria during Pregnancy

Women develop increasing resistance to malaria infection over successive pregnancies. This pattern of parity-specific resistance has been associated with the acquisition of antibodies to the surface of placental parasitized erythrocytes. Early in the pregnancies, women generally lack antibodies that react with the surface of placental binding parasitized erythrocytes, which suggests these express novel surface variants. However, by the second trimester (~ 20 weeks) many primigravid women possess antibodies that react to laboratory-adapted chondroitin sulphate A binding lines, suggesting they have been exposed to placental adherent parasitized erythrocytes (Ricket *et al.,* 2003). Consistent with this interpretation, the blood circulation opens up to the placenta about 10 weeks into pregnancy; and biochemical evidence demonstrates that low sulphate chondriotin sulphate proteoglycans are present in the placenta and intervillous blood spaces by the end of the first trimester and can support parasitized erythrocytes binding in vitro (Agbor- Enoh *et al*., 2003). Binding phenotype suggests that most placental parasites do not undergo a full cycles of replication in the placenta but

circulate and sequester during the later developmental stages. Therefore, women are susceptible to placental infections early in pregnancy, beginning at approximately 10- 12 weeks. During this first exposure, women begin to develop antibodies to placental binding isolates (Brabin, 1996). The peak prevalence of puerperal blood parasitaemia in pregnant women occurs at the beginning of the second trimester (between 13 and 20 weeks gestation). Monocytes and macrophages are commonly seen in infected placenta. They frequently contain malaria haemozoin pigment. After parasites and monocytes clearance, free malaria parasites or fibrin deposits still persist in the placental infections could be classified into four: no infection, acute, chronic and past infection. This grade of classification is based on the presence or absence of PEs and malarial pigment in the placental blood spaces. These grades are believed to reflect a natural progression of infection and immunity. The presence of parasitized erythrocytes and minimal pigment in macrophages but not in fibrin causes acute infections. Chronic infections are characterized by parasitized erythrocytes and pigment deposits in monocytes and fibrin. While past infections have pigment deposites, but no parasitized erythrocytes. The presence of malaria pigment in monocytes intervillous is associated with poor pregnancy outcomes. Therefore, monocytes play a role in both malaria complications and resolution of infection (Duffy *et al.,* 2006).

Studies have shown that both primigravid and multigravid women have adhesion-blocking antibodies effective against chondroitin sulphate A binding isolates. However, the major difference

between primigravid and multigravid women is the timing at which these antibodies are first detected during pregnancy. Thus antibody response may be rapidly boosted upon re-exposure to placental isolates in multigravid women. It has been proposed that this may be a factor in improved pregnancy outcome in multigravid women (Beeson *et al*., 2000). Specific antibodies to the surface of chondroitin sulphate A binding parasites usually develop during pregnancy. These antibodies are low or absent in children or men. Therefore non immune IgG/IgM which facilitates immune evasion is of low affinity. They could be displaced once specific antibodies develop against the chondroitin sulphate A - ligand in multigravid women (Elliott *et al.,* 2005).

2.5 Antenatal Care Services in Nigeria and Malaria in Pregnancy

Each year, thirty million pregnancies are threatened by malaria in endemic countries throughout Africa (Balogun, 2007). In Nigeria, the disease accounts for about 25% infant and 11% maternal mortality (WHO, 2010). Despite the tragedy and economic loss reflected by these percentages, majority of pregnant women in Nigeria do not have access to antenatal services (Idowu *et al.,* 2007). Antenatal care refers to the professional services given to pregnant women to promote and maintain the good health of the expectant mother and the unborn child till safe delivery of a mature and healthy baby. The duration of pregnancy from the moment of the egg is fertilized till labour is about 266 days. Since it is not easy to pinpoint the exact day of fertilization and since most women will not know that they are pregnant till the next

menstrual cycle is missed, the first day of the last cycle is used in calculating the expected date of delivery (Kuti *et al.,* 2006).

For most part of the pregnancy, the woman is monitored to:

i. Detect previously undiagnosed diseases like heart disease, hypertension, diabetes mellitus and renal problems.
ii. Detect, and if possible, prevent complications of pregnancy like anaemia, cephalopelvic disproportion and retardation of foetal growth.
iii. Manage the discomfort and disorders of pregnancy like vomiting and heartburn.
iv. Prepare the woman for labour, lactation and appropriate care of the child.

In the developed world, 97% of women receive prenatal care. This contrast sharply with the experience in many developing countries where less than 30% of the women receive antenatal care (Nwogu, 2009).

Antenatal care may be grouped into two phases: initial and subsequent visits to the health centre. Initial visit is also called booking visit. Ideally, booking should occur not less than 18 weeks of gestation so that appropriate interventions can be implemented where risk factors are indicated (Nwogu, 2009). Activities during the booking visit include obtaining the mother's medical history, physical examination and carrying out further investigations. After booking visit, the frequency of subsequent visits depends on the history of the pregnancy. In the absence of specific risk factors, the expectant mother is recommended to

come for prenatal visit:

i. Every four weeks till 28 weeks
ii. Every two weeks till 36 weeks
iii. Every week till the commencement of labour.

At each visit, the weight, blood pressure and urine test are measured or done. For most women, about 10kg is gained during pregnancy. From the 24th week of pregnancy, mothers should be asked of foetal movement. The haemoglobin is repeated at 30 and 36 weeks of pregnancy. Also at 36 weeks and thereafter, the engagement of the foetal head should be checked (Niganda and Romero, 2003). Antenatal care service if well implemented should provide the simple technologies that exist to prevent and control malaria in pregnancy. These technologies include long lasting insecticide-treated nets (LLINs), which form protective barriers between mosquitoes and women while they sleep; intermittent preventive treatment in pregnancy (IPTp), the drug regimen recommended for protecting women and their unborn babies from the effect of malaria (Jhpiego, 2005).

Antenatal care services increase mother's chances to stay alive and give birth to a healthy baby. This is achieved by providing special care and monitoring to pregnant women before birth. Pregnancy if not cared for is always a risk to the mother especially in complicated cases (Ogbonnaya *et al.,* 2005). Maternal mortality occurs because of complications during pregnancy and delivery. But some of these can be noticed in antenatal care before they become life threatening. Unfortunately, there is low overall utilization of antenatal care services among Nigeria women

compared to women in other African countries (Sule-Odu, 2000). According to Dairo (2010), three main factors affecting the utilization of antenatal care services include inability to afford cost of antenatal care; the long time that will be spent in obtaining antenatal care; and ignorance to importance of antenatal.

The antenatal care services in Nigeria, Malaria Action Coalition (MAC) in collaboration with National Malaria Control Program of the Federal Ministry of Health, Roll Back Malaria (RBM) partners and other key stakeholders have develop policies and guidelines that aim at improving the overall health status of both mother and child. The following are the ideal antenatal services provided for pregnant mother:

i. Education activities aimed at developing orientation package, producing complete curriculum and training package; conducting workshops and trainings.
ii. Measuring the size of the belly by tape measure.
iii. Palpation – Examining the belly with hands or fingertips (the ultrasound system is used in some hospitals).
iv. Checking the blood pressure, blood group and genotype.
v. Haemoglobin test to determine percentage of blood in the body.
vi. Urine test to detect the level of blood sugar and protein.
vii. Administration of iron tablets and folic acids.
viii. Screening for Sexual Transmitted Infections (STIs), Tuberculosis (TB) and Human Immunodeficiency Virus/Acquired Immune Deficiency Syndrome (HIV/AIDS).
ix. Tetanus toxoid vaccination.

x. Malaria prophylaxis (IPTp).

xi. Counselling on the signs of danger in pregnancy, safer sex and contraceptive.

xii. Health education on nutrition, body fitness and breast feeding (FHI, 2004).

All the antenatal clinics use antenatal care cards. This helps in controlling the amount of visits. Also, information on the progress of pregnancy is marked on these cards. The card also contains the possible next antenatal date visit depending on the age of pregnancy. HIV positive mothers are given special birth planning during antenatal (CDC, 2002).

2.6 Confirmatory Diagnosis of Malaria

Confirmatory diagnosis of malaria involves identification of malaria parasite or its antigens/products in the blood of the patient. Although this seems simple, the efficacy of the diagnosis is subject to many factors (Redd *et al.,* 1996). These include different forms of the four malaria species; the different stages of erythrocytic schizogony; the endemicity of different species; the population movements; the inter-relation between the levels of transmission, immunity, parasitaemia, and the symptoms; the problems of recurrent malaria, drug resistance, persisting viable or non-viable parasitaemia, and sequestration of the parasites in the deeper tissues; and the use of chemoprophylaxis or even presumptive treatment on the basis of clinical diagnosis can all have a bearing on the identification and interpretation of malaria parasitaemia on a diagnostic test (Redd *et al.,* 1996; Olivar *et al.,*

1991). The diagnosis of malaria is confirmed by blood tests and can be divided into microscopic and non-microscopic tests.

2.6.1 Microscopic Tests

For nearly a hundred years, the direct microscopic visualization of the parasite on the thick and/or thin blood smears has been the accepted method for the diagnosis of malaria in most settings, from the clinical laboratory to the field surveys. Light microscopy of thick and thin stained blood smears remains the standard method for diagnosing malaria. It involves collection of a blood sample, its staining with Giemsa or alternative stains and examination of the Red Blood Cells for intracellular malarial parasites. Thick smears are 20–40 times more sensitive than thin smears for screening of *Plasmodium* parasites, with a detection limit of 10–50 trophozoites/µl (Lopez-Antunano, 1990). Thin smears allow one to identify malaria species (including the diagnosis of mixed infections), quantify parasitaemia, and assess for the presence of schizonts, gametocytes, and malarial pigment in neutrophils and monocytes (Lopez-Antunano, 1990). The diagnostic accuracy relies on the quality of the blood smear and experience of laboratory personnel.

Before reporting a negative result, at least 200 oil immersion visual fields at a magnification of 100× should be examined on both thick and thin smears, which have a sensitivity of 90% (Gilles, 1993). The level of parasitaemia may be expressed either as a percentage of parasitized erythrocytes or as the number of parasites per micro litre of blood. In non-*falciparum* malaria, parasitaemia rarely exceeds 2%, whereas it can be considerably

higher (>50%) in *falciparum* malaria (Lopez-Antunano, 1990). In non-immune individuals, hyper parasitaemia (>5% parasitaemia or >250 000 parasites/µl) is generally associated with severe disease (Lopez-Antunano, 1990).

In *falciparum* malaria, parasitized erythrocytes may be sequestered in tissue capillaries resulting in a falsely low parasite count in the peripheral blood ('visible' parasitaemia). In such instances, the developmental stages of the parasite seen on blood smear may help to assess disease severity better than parasite count alone. The presence of more mature parasite forms (>20% of parasites as late trophozoites and schizonts) and of more than 5% of neutrophils containing malarial pigment indicates more advanced disease and a worse prognosis (Gilles, 1993). One negative blood smear makes the diagnosis of malaria very unlikely (especially the severe form); however, smears should be repeated every 6–12 hours for 48 hours if malaria is still suspected (Gilles, 1993)

The smear can be prepared from blood collected by venipuncture, finger prick and ear lobe stab. In obstetric practice, cord blood and placental impression smears can be used. In fatal cases, post-mortem smears of cerebral grey matter obtained by needle necropsy through the foramen magnum, superior orbital fissure, ethmoid sinus via the nose or through fontanel in young children can be used. Sometimes no parasites can be found in peripheral blood smears from patients with malaria, even in severe infections. This may be explained by partial antimalarial treatment or by sequestration of parasitized cells in deep

vascular beds. In these cases, parasites, or malarial pigment may be found in the bone marrow aspirates. Presence of malarial pigment in circulating neutrophils and monocytes may also suggest the possibility of malaria.

2.6.2 Rapid Diagnostic Tests (RDTs)

Although the peripheral blood smear examination that provides the most comprehensive information on a single test format has been the "gold standard" for the diagnosis of malaria, the immunochromatographic tests for the detection of malaria antigens, developed in the past decade, have opened a new and exciting avenue in malaria diagnosis. Immunochromatographic tests are based on the capture of the parasite antigens from the peripheral blood using either monoclonal or polyclonal antibodies against the parasite antigen targets. Currently, immunochromatographic tests can target the histidine-rich protein 2 of *P. falciparum* (PfHRP2), a pan-malarial *Plasmodium* aldolase, and the parasite specific lactate dehydrogenase (pLDH) (Moody, 2002). These RDTs do not require a laboratory, electricity, or any special equipment.

PfHRP2 is a water soluble protein that is produced by the asexual stages and gametocytes of *P. falciparum,* expressed on the red cell membrane surface, and shown to remain in the blood for at least 28 days after the initiation of antimalarial therapy. *Plasmodium* aldolase is an enzyme of the parasite glycolytic pathway expressed by the blood stages of *P. falciparum* as well as the non-*falciparum* malaria parasites. Monoclonal antibodies against *Plasmodium* aldolase are pan-specific in their reaction and have

been used in a combined 'P.f/P.v' immunochromatographic test that targets the pan malarial antigen (PMA) along with PfHRP2 (Moody, 2002). Parasite lactate dehydrogenase (pLDH) is a soluble glycolytic enzyme produced by the asexual and sexual stages of the live parasites and it is present in and released from the parasite infected erythrocytes. It has been found in all 4 human malaria species, and different isomers of pLDH for each of the 4 species exist. With pLDH as the target, a quantitative immunocapture assay, a qualitative immunochromatographic dipstick assay using monoclonal antibodies, an immunodot assay, and a dipstick assay using polyclonal antibodies have been developed.

The RDTs have been developed in different test formats like the dipstick, strip, card, pad, well, or cassette; and the latter has provided a more satisfactory device for safety and manipulation (Lee *et al*., 2002). The test procedure varies between the test kits. In general, the blood specimen (2 to 50μL) is either a finger-prick blood specimen, anticoagulated blood, or plasma, and it is mixed with a buffer solution that contains a haemolysing compound and a specific antibody that is labelled with a visually detectable marker such as colloidal gold. In some kits, labelled antibody is pre-deposited during manufacture and only a lysing/washing buffer is added. If the target antigen is present in the blood, a labelled antigen/antibody complex is formed and it migrates up the test strip to be captured by the pre-deposited capture antibodies specific against the antigens and against the labelled antibody (as a procedural control). A washing buffer is then added to remove the haemoglobin and permit visualization of any

coloured lines formed by the immobilized antigen-antibody complexes. The pLDH test is formatted to detect a parasitaemia of >100 to 200 parasites/μL and some of the PfHRP2 tests are said to detect asexual parasitaemia of >40 parasites/μL (Lee *et al*., 2002).

The PfHRP2 test strips have two lines, one for the control and the other for the PfHRP2 antigen. The PfHRP2/PMA test strips and the pLDH test strips have three lines, one for control, and the other two for *P. falciparum* (PfHRP2 or pLDH specific for *P. falciparum)* and non-falciparum antigens (PMA or pan specific pLDH), respectively. Change of colour on the control line is necessary to validate the test and its non-appearance, with or without colour changes on the test lines, invalidates the test. With colour change only on the control line and without colour change on the other lines, the test is interpreted as negative. With the PfHRP2 test, colour change on both the lines is interpreted as a positive test for *P. falciparum* malaria. With the PfHRP2/PMA [the immunochromatographic test (ICT Malaria P. f. /P.v.test)] and the pLDH tests, colour change on the control line and the pan specific line indicates non-fa1ciparum infection and colour change on all the 3 lines indicates the presence of *P. falciparum* infection, either as mono-infection or as a mixed infection with non-falciparum species. Also, if the PfHRP2 line is visible when the PMA line is not, the test is interpreted as positive for *P. falciparum* infection. Mixed infections of *P. falciparum* with the non-falciparum species cannot be differentiated from pure *P. falciparum* infections. However, with regard to the pLDH test, it is claimed that in the presence of *P.*

vivax infection, the genus specific line is much darker and more intense than the species specific line due to the presence of all the stages of the parasite in the blood (Hanscheid and Grobusch, 2002).

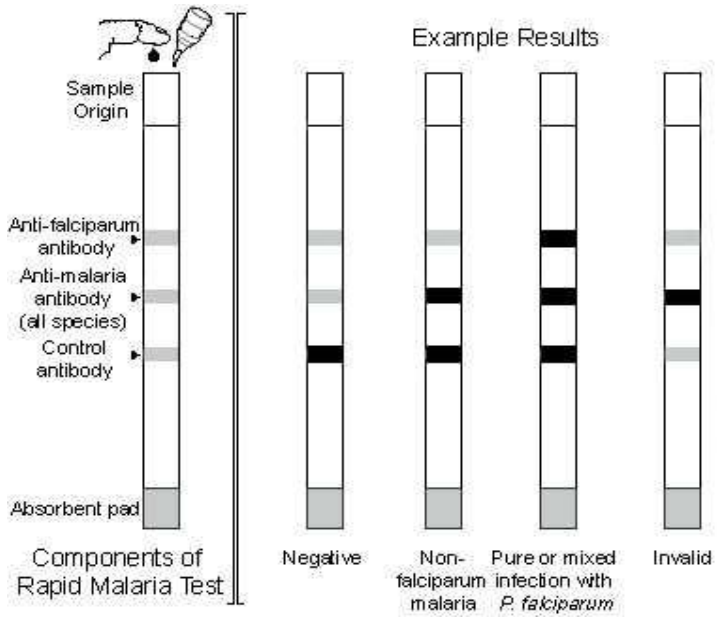
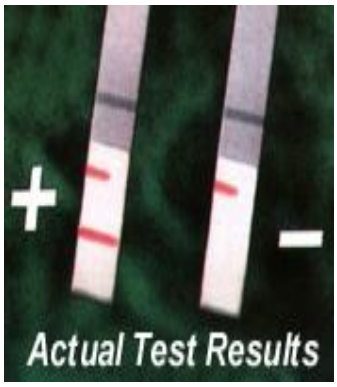


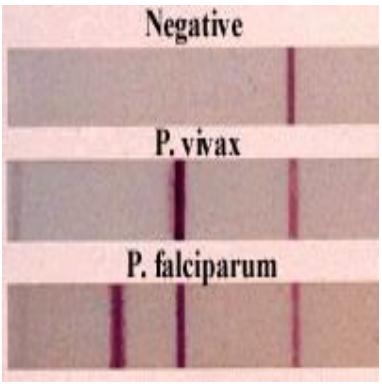
Figure 1: RDT Test Format

Source: http://cmr.highwire.org/cgi/content/full/15/1/66



Para Sight F test

OptiMal Assay Kit



OptiMal Assay Result

Figure 2: Examples of RDTs

Source: http://cmr.highwire.org/cgi/content/full/15/1/66

2.7 Challenges to Diagnosis of Malaria

Access to medical care is limited in many malaria-endemic areas and where medical services exist, they commonly lack facilities for laboratory diagnosis, and as a result, malaria treatment is mostly given on the basis of clinical or self diagnosis (Chiodini, 1998). Determination of a patient's clinical history and symptoms is an acceptable basis for the management of malaria disease (WHO 2000a). Although the signs and symptoms of malaria, such as fever, chills, headache and anorexia, are generally non-specific, some signs and symptoms, especially in combination, have diagnostic value in specific epidemiological and operational situations (Redd *et al*., 1996). However, it is not possible to apply any one set of clinical criteria to the diagnosis of all types of malaria in all patient populations. Experience has shown that the appropriateness of particular clinical diagnostic criteria vary from area to area according to the intensity of transmission, the prevalent malaria species, the incidence of other causes of fever, the qualifications of the health care staff and the health service infrastructure (WHO, 2000a).

The observation of fever alone, and of fever in combination with chills and/or headache, achieved quite high sensitivities, but both criteria resulted in high rates of overtreatment and any narrower combination of symptoms resulted in sensitivities unacceptable in relation to the detection of a life-threatening illness, yet, measurement of axillary temperature failed to achieve sufficient sensitivity or specificity to be useful (Collins and Jeffery, 1999). In areas where malaria is endemic, clinical

diagnosis usually results in all patients with fever and no other apparent causes of malaria being treated for malaria (WHO, 2000a). This approach can identify most patients that really need malaria treatment but is also likely to misclassify many who are not. The specificity of clinical diagnosis is only 20-60% compared with microscopy (Redd *et al*, 1996; WHO, 2000a). While clinical diagnosis offers the advantages of ease, speed and low cost, over-diagnosis can be substantial and contributes to the misuse of antimalarial drugs (WHO, 2000a). Clinical diagnosis is very inaccurate, even in areas where malaria is a common cause of fever, since signs and symptoms of uncomplicated malaria are non-specific and overlap with those of other febrile infectious diseases (Redd *et al*, 1996) and the subjective sensation of fever is unreliable (WHO, 2000a).

Given the low specificity of all clinical case definitions, there is a compelling need to make parasite detection more widely available. Evidence of the presence of parasites can be made by the examination of a stained blood smear by light microscopy. Basic microscopy has the advantages of low direct costs if the infrastructure to maintain the service is already available (WHO, 2000a); can be sensitive if the quality of microscopy is high (Redd *et al*, 1996); can be used to differentiate between species; determine parasite densities (Redd *et al*, 1996); and can be used to diagnose many other conditions.

However, experience in malaria endemic areas has shown that it can be difficult to maintain good microscopy at the periphery of the health services where most patients are treated because of

the poor quality of microscopists, particularly at the peripheral level (Tarimo *et al*., 2001); difficulties in maintaining microscopical facilities in good order; logistic problems and high costs of maintaining adequate supplies and equipment; lack of adequate training and retraining of laboratory staff; delays in providing results to clinical staff; and lack of quality assurance and control of laboratory services (WHO, 2000a). Still, in areas with intense transmission, it is of limited value for children and to some extent for adults, as asymptomatic parasite carriers may be common (WHO, 2000a). However, in these areas, the WHO Expert Committee recommended that confirmatory diagnosis is desirable to detect treatment failures, confirm severe disease, and diagnosing complicated malaria during a low transmission season (WHO, 2010).

When parasite-based diagnosis is essential, Rapid Diagnostic Test (RDT) may be an alternative to light microscopy in situations where normal laboratory services are non-existent or overworked. A review of the current evidence on the use of RDTs by WHO informal consultations (WHO, 2003) identified that these tests have many potential advantages. It produces rapid results, which is very useful in clinical care as well as rapid epidemiological assessments; needs a lower level of training/skilled personnel; requires lower capital costs than light microscopy (but they can be more costly when case numbers are high); reinforces patient confidence in the diagnosis and the health service; also identifies patients that do not have malaria for which another source of fever should be sought and permits rational use of high-cost drugs, thus reducing costs in areas where artemisinin-

combination therapy is required for *P. falciparum* based solely on clinical diagnosis. This may not apply if parasite prevalence is very high, in which case the additional costs of improved diagnosis may provide little benefit in terms of savings on drug costs. On the other hand, where prevalence (and host immunity) is high, RDT test results may erroneously suggest a positive diagnosis in patients with parasitaemia incidental to another illness (WHO, 2003). RDTs detect antigens and not parasites, results may therefore reflect recent and not current parasitaemia. Nevertheless, antigen detection may be a better indication of parasite load than light microscopy (WHO, 2003). Sensitivity in the field may be unpredictable. Published sensitivities for *P. falciparum* range from comparable to good field microscopy (>90 % at 100-500 paratsites/µl) to very poor (40-50 %) for some widely used products. Sensitivities are generally lower for non-falciparum species (WHO, 2003). Reasons for poor sensitivity are not apparent. They may be attributable to poor manufacture, damage due to temperature or humidity exposure, incorrect handling by end-users, possible geographical variation in the test antigen and poor comparative microscopy (WHO, 2003).

2.8 Knowledge about Symptoms of Malaria

Federal Ministry of Health (FMOH) survey assumed that the households had good knowledge of the symptoms of malaria if they mentioned at least fever, headache, chill and joint pain but poor knowledge if they mentioned fever plus general weaknesses or dizziness. In Ogun (urban Ado-Odo), 78% of households were considered to have good knowledge of the symptoms of malaria

as compared with 49% in the rural area of Ado-Odo (Oreagba *et al*., 2004). According to studies by Adedotun *et al.*, 2010 and Oreagba, 2004, knowledge about signs and symptoms of malaria is relatively high with most respondents indicating awareness of key symptoms including raise in temperature/hot body followed by other symptoms like vomiting, loss of appetite and restlessness. A study done by Adedotun *et al.,* (2010) in Oyo indicated that caregivers had a good understanding of how to recognize malaria, with 91% reporting high body temperature, 50% headache, 25% body pain, 23% chills, 45% vomiting and 74% poor appetite. However, in the same study, the understanding and recognition of severe malaria was very low among the caretakers, with only 11% mentioning convulsions as a sign of severe malaria, 25% lethargy/weakness, and 26% anorexia. While general knowledge of malaria symptoms is relatively high, reviewed research indicates that symptoms of severe malaria are not well known among community members (Fapohunda, 2004; Njama, 2003). The less common symptoms, which require close observation and medical interpretation like jaundice, anaemia and splenomegally, were also not well known (Njama, 2003). Convulsions as a sign of severe malaria were not widely mentioned by the respondents in most studies reviewed. Onyeneho (2006) makes similar observations based on a study in Imo state that mothers (as caretakers) appeared not to easily recognise anaemia unless told by health workers. In the same study, it is indicated that although altered consciousness and convulsions were recognised signs (by some caretakers) that the child was unwell, they were often thought to be traditional

diseases best managed by traditional means. Onyeneho (2006) also reports that although splenemogally was fairly recognised by elderly women, most mothers did not think splenemogally was due to malaria, instead they thought that splenemogally caused malaria. The limited knowledge of signs of severe malaria, including convulsions, indicates an area that requires strengthening largely through health education and communication (Fapohunda *et al.*, 2004).

2.9 Attitude towards malaria

Community members' attitude towards malaria as a disease is important in understanding their health seeking behaviour. Some of the studies reviewed have indicated that communities now regard malaria as a dangerous disease that can kill and affects more children under five years than the adults. Studies reviewed also indicate that most community members strongly felt that malaria can be prevented. Such positive attitudes are essential opportunities for behaviour change campaigns. In a study done in Ibadan (Erhun, 2005), 87.5% of the community knew that malaria can be prevented while others thought otherwise. In a study in Mbaise, Imo state, it is indicated that 72% of the community members thought that malaria can be prevented (Onyeneho, 2006). The Commercial Market Strategies (CMS) survey (Adedotun *et al.*, 2010) in districts of Ogun noted that majority of respondents (98%) believed that malaria was dangerous and could cause death. A study done by Erhun (2005) in southwestern Nigerian community indicates 95.4% of respondents looked at malaria as a severe problem that could

kill. Other qualitative studies have indicated that malaria in pregnancy is a normal thing (Kengeya, 2004; Nuwaha, 2002).

Other studies have also indicated that communities generally observed that malaria was a seasonal problem in their areas (Agu and Nwojiji (2005). While malaria is seasonal in most parts of the country, this perception could be linked to inconsistent use of malaria preventive methods as reported in most of the studies reviewed. The CMS survey (Twebaze, 1998) noted a strong perception among community members (75%) that children under five were more vulnerable to malaria than older children or adults; and only 10% of the respondents felt that pregnant women were also more vulnerable to malaria. In the same survey, no major differences were observed between rural and urban areas.

2.10 Gender Roles and Treatment Seeking Behaviour

Gender relations are one of the reasons why mothers as caretakers do not seek early formal malaria treatment (Twebaze (1998). Mothers made most of the decisions regarding home treatment of malaria including assessing the symptoms and diagnosis (Aina, 2005). Bakika (1994) noted that first course of treatment at home largely involved decisions by mothers and caretakers, while second level treatment outside the home involved decisions of fathers and male guardians. Women in most cases have to seek for permission from their husbands or some have to first be supported financially by the husbands before seeking for treatment outside the home. Twebaze (1998) show that most fathers only got involved in the second and third stages

of malaria treatment, and when there were monetary considerations to be met. Elderly women such as mothers-in-law, grandmothers, often advised mothers/caretakers about the use of herbs and supportive treatment like wet sponging.

Treatment seeking behaviour has been shown to be related to the cost, availability and cultural beliefs about the causes and effective cures for malaria-like symptoms (Chuma *et al.,* 2010). Self medication with antimalarial drugs has been reported to be a common practice in many endemic areas worldwide (Yeneneh *et al.*, 1993). This may likely have a financial undertone as well as availability and affordability of sorts of antimalarial in drug stores (Mwenesi *et al*., 1995). Cultural and social factors have been reported to influence treatment-seeking behaviour (Mwenesi *et al*., 1995). It was deduced from the participant's response that wrong medication, incomplete doses, late or improper diagnosis, pricing and availability of genuine antimalarial drugs are some of the factors militating against proper management of the illness and in most cases responsible for relapses and recrudescence of the infection (Mwenesi *et al*., 1995; Yeneneh *et al.*, 1993). According to Alba *et al.*, (2001) early diagnosis and treatment of malaria is well recognized as a strategy for reducing morbidity and mortality of the disease.

2.11 Knowledge and Attitude towards Diagnosis

In many rural endemic areas, clinical diagnosis of malaria is highly practiced, as access to microscopic examination of the parasite is limited (Njama, and Dorsey, 2003; Stow *et al*., 1999). In a community interviews on the three alternative diagnostic

strategies done by Okanurak and Ruebush (2006), just 1% of the respondent would not use any intervention based on the health service, 15% preferred slide, 48% chose RDTs and 36% treated malaria based on signs and symptoms only. Most (63%) preferred investigation that involved some form of blood sampling. Rapid diagnostic tests were easily the most popular choice, despite the cost, which was roughly half the minimum true cost of the tests (Okanurak and Ruebush, 2006; Goodman *et al*., 1999; Premji, 1994). Delayed slide-based diagnosis was preferred to unconfirmed symptom-based diagnosis (Okanurak and Ruebush, 2006).

In a comparative study on acceptance of different malaria diagnosis by Bell *et al.,* 2001, 100% of the community health workers interviewed considered that it was advantageous to obtain immediate diagnosis by means of the immuno-chromatographic tests (ICT). In that same study, delayed slide-based diagnosis was considered of some use by 85% of the health workers questioned, although 26% expressed reservations about late receipt. 19% modified their normal practice by including closer follow-up of positive cases and 11% did so by either withholding treatment or withdrawing treatment because of side-effects after negative results. 11% searched harder for alternative diagnoses after negative results. 26% health workers mentioned that a preference among patients for ICT tests led to increased treatment-seeking behaviour and compliance. Some mentioned increases in job satisfaction, their standing in the community, and their clinical knowledge.

Community attitudes to diagnosis demonstrated the importance of providing patients with a reliable explanation for their illness. An immediate blood based diagnosis at some cost was preferred by community members to both delayed free slide diagnosis and symptom-based diagnosis (Okanurak and Ruebush, 2006; Goodman *et al*., 1999; Premji, 1994), despite the cost to the patient. This cost, approximately the difference between the estimated cost of microscopy to the health service and the bulk wholesale cost of the rapid diagnostic tests, was a substantial sum to families engaged in semi subsistence agriculture (Premji, 1994). The cost-effectiveness of symptom-based diagnosis, rapid diagnostic tests and microscopy, and the proportion of costs that the community is willing to bear vary with transmission rates and health-service access (Okanurak and Ruebush, 2006). In the long term, improved compliance and treatment-seeking behaviour may bring additional economic benefits from rapid diagnostic tests through a reduced burden of illness (Pagnoni, 1997). The detection of persistent antigen in asymptomatic infection, when fluctuating parasitaemia reduces the sensitivity of microscopy (Collins and Jeffery, 1999; Roper *et al*., 1996), also offers new possibilities for rapid screening of communities at risk. In a study by Roper *et al.,* 1996 clearly showed that malarial parasitaemia cannot be easily identified by symptoms alone and that microscopy was unreliable in remote areas. In that same study, the rapid diagnostic test was well accepted by community volunteers and was performed accurately by them after little training. It markedly improved diagnostic accuracy and met a desire in the community for rapid blood-based diagnosis.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study was conducted in Ozubulu community in Ekwusigo Local Government Area (LGA) of Anambra State, southeast Nigeria. Ozubulu is the headquarters of Ekwusigo LGA and the most populated community among the four communities that make up the LGA. The community is predominantly rural with a population of approximately 46,062 (24,234 males and 21,828 female) and a population density of up to 600/km^2 (NPC, 2006). The geographical coordinates of Ozubulu are 5.57^{0}N latitude, 6.51^{0}E longitude and 470 feet above sea level. According to Ilika (2007), Ozubulu is within the rainforest and stable malaria transmission belt of Anambra State. It experiences two distinct seasons - a wet season of abundant rainfall which begins in April and ends in October or early November, and a dry season which lasts from November to March. There are two rainfall peaks, one in July and the other in September, and annual precipitation is in excess of 2000mm (Okwa *et al.,* 2009). Temperature is high throughout the year with day time range of 23^{0}C - 35^{0}C. Malaria is endemic in these areas and occurs throughout the year with peaks during the rainy season (Ilika, 2007).

Ozubulu shares boundaries in the north with the towns of Nnewi and Oraifite, in the south with Ihembosi and Okija, in the west with Atani, and in the east with Ukpor. Ozubulu is made of four villages

namely: Amakwa, Egbema, Eziora and Nza. The community members are predominantly Christians, with little practicing traditional religion. The tribe of this community is Igbo. The local economy is based on agriculture and trading, with a small proportion of the population working in private and public sectors (FMOI, 2008). Ozubulu has four market days: Afor, Nkwo, Eke, and Orie, which is its biggest market day.

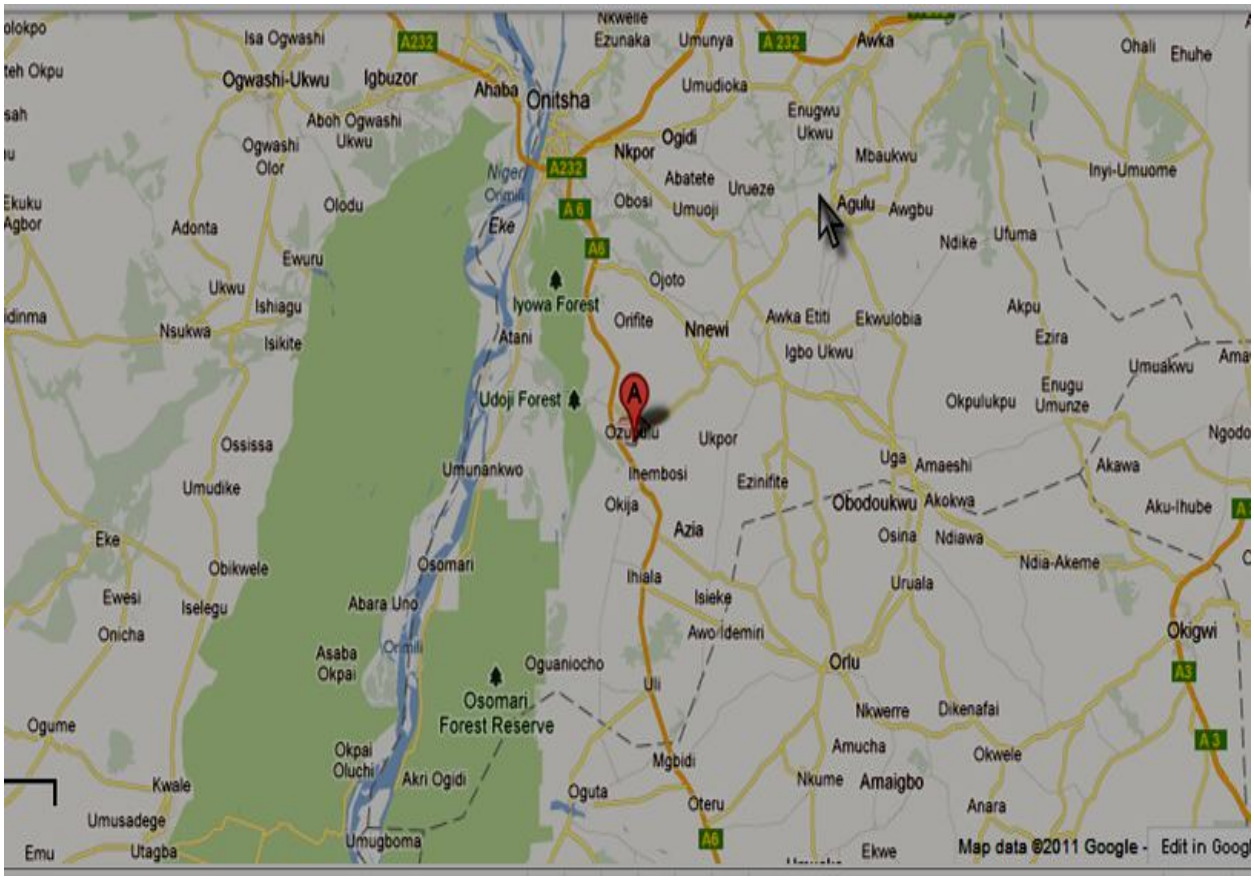


Figure 3: Map of Anambra region of Nigeria showing the study area - Ozubulu

Scale: 1.5cm : 10km
Source: http://maps.google.com.ng/maps
Accessed: 1/11/2011

3.2 Sample Population

The sample population for prevalence of malaria was pregnant women that attended ante-natal clinic in hospitals in Ozubulu. For knowledge and attitude to malaria diagnosis, the sample population was caregivers from selected households in the community.

3.3 Sample Size

A total of 243 pregnant women were sampled for prevalence of malaria. To arrive at the sample size, the annual 3% growth rate for the female population was determined as at 2011. The population of women of reproductive age, which is 49% of all female population, was determined. Also determined was the population of pregnant women; who constitute 5% of women of reproductive age in Nigeria. The figure obtained was substituted in the formula for determining sample size: $n = N/1+N (e^2)$; where n = minimum sample size, N = total population to be sampled, e = error term at 5% (95% confidence interval) (Rao and Scott, 1992).

For knowledge and attitude to diagnosis, a total of 393 caregivers were sampled. To arrive at the sample size, the annual 3% growth rate for the total population was determined as at 2011. The adult population, which is 45% of total population, was also determined. The figure obtained was substituted in the formula for determining sample size.

3.4 Permission Obtainment

A letter of Identification from the Department of Parasitology and Entomology, Nnamdi Azikiwe University, Awka was used in pre-

survey visits made to the hospitals to obtain permission from the hospitals' authorities. During the visits, the management, health workers in charge of antenatal services and laboratory scientists were informed on the nature and objectives of the study. They later organized and informed the pregnant women about the study. The informed consent of both management and the pregnant women were sought and obtained before the commencement of the study.

Oral consent of both the traditional ruler of Ozubulu and the caregivers were sought and obtained before the commencement of the study in knowledge and attitude of community members to malaria diagnosis.

3.5 Research Design

The research consists of two parts. The first part was on prevalence of malaria in pregnant women attending antenatal clinic. The second part was on knowledge and attitude of community members on diagnosis of malaria.

3.5.1 Method for Investigating the Prevalence of Malaria in Pregnancy

The study was a hospital based cross-sectional survey conducted between November 2011 and January 2012. Four hospitals (57%) namely: Joint Hospital, Evans specialist Hospital, Layola Specialist Hospital and, St. Jude Hospital and Maternity were selected out of a total of seven hospitals in this community using simple random sampling. The 243 pregnant women selected were women of reproductive age (15 – 45 years). Peripheral blood samples were

collected from pregnant women in each of the hospitals once a week, during the antenatal visits. This lasted for three months. The consent of the patients was taken before commencing the study.

Questionnaires concerning age, gravidity, pregnancy stage, level of education and occupation were distributed to the sampled pregnant women, and with my assistance and that of some of the nurses, the questionnaires were completed by the sampled pregnant women. Blood samples collected were preserved with Ethylenediaminetetraacetic acid (EDTA) before being transported to the laboratory of Parasitology and Entomology of the Nnamdi Azikiwe University, Awka, for analysis.

Thick and thin blood films as described by Cheesbrough (1998) were made on clean slides and labelled accordingly as recommended by WHO, 2002. To prepare the thick film, I placed 2 drops of blood sample on a slide using a small rubber pipette. The blood was gently mixed for 20 seconds using the corner of a second slide to defibrinate the blood and to obtain a round smear. The slide was immersed in the staining trough, containing Giemsa solution prepared with buffered water in the ratio of 1:20, and contact was maintained for 30-40 minutes, and then allowed to dry.

For the thin film, I placed a drop of blood sample on a clean slide using a rubber pipette. The edge of a second slide was then laid on the drop of blood that would spread on the entire line of contact between the two slides. The second slide, steadily held by me to

form a 45° angle with the original slide was then moved to the opposite end of the slide to which the drop was originally located. The thin film was fixed using Methanol (methyl alcohol) by maintaining contact with methanol for 10 seconds. The slide was immersed in the staining trough containing Giemsa solution prepared with buffered water in the ratio of 1:10, and contact was maintained for 30 minutes, and then allowed to dry. Stained slides were examined under the light microscope using ×100 objective lens (immersion oil). The thick films were to identify the parasite densities while thin films were used to identify the parasite species.

3.5.2 Observations on Knowledge and Attitude of Caregivers to Malaria Diagnosis

The study was a community based cross-sectional survey conducted between November 2011 and January 2012. First of all, names of all kindreds in each village were listed and five kindreds from each village were selected using simple random sampling, making a total of twenty kindreds. In the second stage, twenty households were selected from each kindred using systematic random sampling to obtain three hundred and ninety three caregivers/interviewees. Data were collected using a pre-tested structured questionnaire specifically developed for determining knowledge, attitude of community members to diagnosis of malaria (Appendix I). Close/structured questionnaire was chosen over the other types as it satisfied the research needs given that it was easy to fill out, took little time, kept the respondents focused on the subject of study, and was fairly easy to tabulate and analyse. A

caregiver was defined as a mother, a father or any adult responsible for the care of a person in a household. This structured questionnaire comprises 10 questions. The questionnaire included two sections. The first section elicited socio-demographic details (age, marital status, educational level, occupation). The second part investigated their knowledge and attitude to malaria and its diagnosis. The questionnaire was pre-tested in Eziora village before the final draft was made. Respondents were interviewed in their homes. It took approximately 15 minutes to administer a questionnaire per person. All participants gave their verbal consent. I used both English and Igbo languages in explaining the content before administering the questionnaire.

3.6 Data Analysis

The data generated on prevalence was analyzed using statistical programme for service solution (SPSS) 17.0 Window versions. The statistical significance of variables was estimated using Chi-square test. Pearson correlation analysis was used to establish possible correlation of prevalence with parity, age, trimester as shown in Appendix III. P-values of equal to or less than 0.05 was taken as measures of significance.

Data on Knowledge and Attitude was analyzed also using SPSS version 17.0 for Window. Cross tabulations of important variables were done and the statistical significance of variables was estimated using Chi-square test.

CHAPTER FOUR

RESULTS

Of the 243 pregnant women whose peripheral blood samples were examined for infection with malaria parasites, 53.9% (131/243) were positive (Table 1). All were infections of *Plasmodium falciparum.*

According to parity, primigravids had the highest infection rate with 65.8% (48/73) being infected. Among the multigravids, the highest infection of 61.1% (22/36) was among the secundigravids. This was followed by women of 3rd pregnancy with prevalence of 51.2% (21/41) and women of 4th pregnancy and above had the least prevalence of 43.0% (40/93). The result is as shown in Table 1. The differences were statistically significant ($P < 0.05$).

Table 1: Prevalence of malaria according to parity status among pregnant women in Ozubulu, Anambra State.

Parity	No. Examined (%)	No. Positive (%)
Primigravid (1st Pregnancy)	73 (30.0)	48 (65.8)
Secundigravid (2nd Pregnancy)	36 (14.8)	22 (61.1)
Multigravid (3rd Pregnancy)	41 (16.9)	21 (51.2)
Multigravid (4th Pregnancy & above)	93 (38.3)	40 (43.0)
Total	**243 (100)**	**131 (53.9)**

* Numbers in parenthesis are percentages.

The result also showed that according to age, pregnant women aged 21-25 years had highest infection rate of 68.8% (33/48), others were 66.7% (24/36) for age group 16-20 years, 63.6% (42/66) for age group 26-30 years, 40.9% (18/44) for age group 31-35 years, 32.4% (11/34) for age group 36-40% years and the least 20.0% (3/15) for age group 41-45 years as shown in Table 2. The differences were statistically significant (P < 0.05).

Table 2: Prevalence of malaria parasite among pregnant women according to age groups in Ozubulu, Anambra State.

Age in years	No. Examined (%)	No. Positive (%)
16 – 20	36 (14.8)	24 (66.7)
21 – 25	48 (19.8)	33 (68.8)
26 – 30	66 (27.2)	42 (63.6)
31 – 35	44 (18.1)	18 (40.9)
36 – 40	34 (14.0)	11 (32.4)
41 – 45	15 (6.2)	3 (20.0)
Total	**243 (100)**	**1313.9)**

* Numbers in parenthesis are percentages

It was also observed that according to gestational age of pregnancy, women of first trimester had the highest prevalence of 71.6% (58/81). This was followed by second trimester 46.3% (31/67) and the least was 44.2% (42/95) for third trimester. The results are as shown in Table 3. The differences were statistically significant (P < 0.05).

Table 3: Prevalence of malaria parasite among pregnant women according to gestational age in Ozubulu, Anambra State.

Gestational age	No. Examined (%)	No. Positive (%)
1st Trimester	81(33.3)	58 (71.6)
2nd Trimester	67 (27.6)	31 (46.3)
3rd Trimester	95 (39.1)	42 (44.2)
Total	**243 (100)**	**131 (53.9)**

* Numbers in parenthesis are percentages.

On knowledge and attitude of caregivers to malaria and its diagnosis, all the 393 caregivers interviewed had heard about malaria and its diagnosis. All caregivers mentioned fever as a sign of malaria. Chills and headaches were mentioned by 86.5% (340/393) and 77.9% (306/393) of the caregivers, respectively. Joints/body pain and weakness were also reported by 54.7% (215/393) and 29.8% (117/393), respectively. Other symptoms mentioned were vomiting and dizziness by 33.3% (131/393) of the caregivers (Table 4).

It was observed also that the highest response of 95.8% (46/48) on chill was seen in age group > 60 years and lowest response of 79.9% (94/118) among age group 31-40 years. Highest response on headache 88.2% (45/51) was seen among age group 51-60 years and the least 68.9% (51/74) among age group 21-30 years. Joints/body pains and weakness were mentioned most by age

groups >60 years (79.2%) and 51-60 years (35.3%), respectively, and the least 28.4% (21/74) and 25.4% (30/118) among age groups 21-30 years and 31-40 years, respectively as shown in Table 4. The differences according to age were not statistically significant (P > 0.05).

Table 4: Knowledge of signs and symptoms of malaria according to ages of caregivers in Ozubulu.

Age in years	No. sampled (%)	Common signs and symptoms of malaria (% response)					
		Chill (%)	Fever (%)	Headache (%)	Joints /body pain (%)	Weakness (%)	Others (%)
21 – 30	74 (18.8)	60 (81.1)	74 (100)	51 (68.9)	21 (28.4)	21 (28.4)	27 (36.5)
31 – 40	118 (30.0)	94 (79.7)	118 (100)	83 (70.3)	62 (52.5)	30 (25.4)	36 (30.5)
41 – 50	102 (26.0)	92 (90.2)	102 (100)	86 (84.3)	60 (58.8)	32 (31.4)	38 (37.3)
51 - 60	51 (13.0)	48 (94.1)	51 (100)	45 (88.2)	34 (66.7)	18 (35.3)	16 (31.4)
>60	48 (12.2)	46 (95.8)	48 (100)	41 (85.4)	38 (79.2)	16 (33.3)	14 (29.2)
Total	**393 (100)**	**340 (86.5)**	**393 (100)**	**306 (77.9)**	**215 (54.7)**	**117 (29.8)**	**131 (33.3)**

* Numbers in parenthesis are percentages.

Also in the signs and symptoms of malaria, chill was the symptom mentioned most by those that attended secondary school (90.1%) and mentioned least by those with no formal education (73.5%). Headache was mentioned most by those that attended tertiary (94.4%) and least by those with no formal education (61.2%). Joints/body pains were mentioned most by those with tertiary education (77.8%) and least by those with no formal (42.9%). Weakness was mentioned most by those with no formal education

(38.8%) and least by those with secondary educaton (25.4%) as shown in table 5. The differences according to educational status were not statistically significant (P > 0.05).

Table 5: Knowledge of signs and symptoms of malaria according to educational status of caregivers in Ozubulu.

Educational status	No. sampled (%)	Common signs and symptoms of malaria (% response)					
		Chill (%)	Fever (%)	Headache (%)	Joints /body pain (%)	Weakness (%)	Others (%)
Informal	49 (12.5)	36 (73.5)	49 (100)	30 (61.2)	21 (42.9)	19 (38.8)	18 (36.7)
Primary	127 (32.3)	111 (87.4)	127 (100)	103 (81.1)	60 (47.2)	41 (32.3)	48 (37.8)
Secondary	181 (46)	163 (90.1)	181 (100)	139 (76.8)	106 (58.6)	46 (25.4)	59 (32.6)
Tertiary	36 (9.2)	30 (83.3)	36 (100)	34 (94.4)	28 (77.8)	11 (30.6)	6 (16.7)
Total	**393 (100)**	**340 (86.5)**	**393 (100)**	**306 (77.6)**	**215 (54.7)**	**117 (29.8)**	**131 (33.3)**

* Numbers in parenthesis are percentages.

On the knowledge of malaria diagnostic methods, the result showed that all the caregivers interviewed knew about self diagnosis. 57% (224/393) of the caregivers knew about microscopy, while only 2.5% (10/393) knew about RDTs (Table 6).

The result also showed that caregivers aged 41-50 years had highest knowledge of microscopy 69.6% (71/102). Others were 56.8% (67/118) for age group 31-40 years, 47.1% (24/51) for age group 51-60 years, 64.6% (31/48) for age group > 60 years, and the least 41.9% (31/74) for age group 21-30 years. The result also showed that age group > 60 years had the highest knowledge of

RDT (6.3%). Others were 2.7% (2/74) for age group 21-30 years, 2.5% (3/118) for 31-40 years, 2.0% (2/102) for 41-50 years, and the least 0% in 51-60 years as shown in Table 6. The differences by age were not statistically significant (P > 0.05).

Table 6: Knowledge of malaria diagnostic methods according to age of caregivers in Ozubulu.

Age in years	No. sampled (%)	Knowledge of malaria diagnostic methods (% response)		
		Self diagnosis (%)	Microscopy (%)	RDT (%)
21 – 30	74 (18.8)	74 (100)	31 (41.9)	2 (2.7)
31 – 40	118 (30.0)	118 (100)	67 (56.8)	3 (2.5)
41 – 50	102 (26.0)	102 (100)	71 (69.6)	2 (2.0)
51 - 60	51 (13.0)	51 (100)	24 (47.1)	0 (0.0)
>60	48 (12.2)	48 (100)	31 (64.6)	3 (6.3)
Total	**393 (100)**	**393 (100)**	**224 (57.0)**	**10 (2.5)**

* Numbers in parenthesis are percentages.

The result also showed that all caregivers with tertiary education knew about microscopy. Others were 69.1% (125/181) of those with secondary education, 45.7% (58/127) of caregivers with primary education and 10.2% (5/49) of caregivers with no formal education. The result also showed that 27.8% (10/36) of caregivers with tertiary education knew about RDTs. None (0.0%) of the caregivers with no formal education, primary education and secondary education knew about RDTs as shown in table 7. The differences were statistically significant (P < 0.05).

Table 7: Knowledge of malaria diagnostic methods according to educational status of caregivers in Ozubulu.

Educational status	No. sampled (%)	Knowledge of malaria diagnostic methods (% response)		
		Self diagnosis (%)	Microscopy (%)	RDT (%)
Informal	49 (12.5)	49 (100)	5 (10.2)	0 (0.0)
Primary	127 (32.3)	127 (100)	58 (45.7)	0 (0.0)
Secondary	181 (46)	181 (100)	125 (69.1)	0 (0.0)
Tertiary	36 (9.2)	36 (100)	36 (100)	10 (27.8)
Total	**393 (100)**	**393 (100)**	**224 (57.0)**	**10 (2.5)**

* Numbers in parenthesis are percentages.

On the degree of importance of malaria laboratory diagnosis, 40.2% (158/393) of the caregivers rated laboratory diagnosis as not important, 47.8% (188/393) as barely important and 12% (47/393) as very important. 42.2% (50/118) of caregivers aged 31-40 years rated laboratory diagnosis as not important. Others were 40.5% (30/74) for age group 21-30 years, 39.2% (40/102) for age group 41-50 years, 39.2% (20/51) for age group 51-60 years, while caregivers older than 60 years of age recorded least 37.5% (18/48). 56.9% (29/51) of caregivers aged 51-60 years rated laboratory diagnosis as barely important. Others were 51.4% (38/74) in age group 21-30 years, 47.9% (23/48) for caregivers older than 60 years of age, 45.1% (46/102) for age group 41-50 years and least 44.1% (52/118) for age group 31-40 years. 15.9% (16/102) of age group 41-50 years rated laboratory diagnosis as very important. Others were 14.6% (7/48) for age groups > 60 years, 13.6% (16/118) for

age group 31-40 years, 8.1% (6/74) for age group 21-30 years and the least 3.9% (2/59) for group 51-60 years as shown in table 8. The observed difference was not statistically significance (P > 0.05).

Table 8: Caregivers assessment of the importance of laboratory diagnosis according to according to age.

Age in years	No. sampled (%)	Degree of importance of laboratory diagnosis of malaria (% response)		
		Not important (%)	Barely important (%)	Very important (%)
21 – 30	74 (18.8)	30 (40.5)	38 (51.4)	6 (8.1)
31 – 40	118 (30.0)	50 (42.4)	52 (44.1)	16 (13.6)
41 – 50	102 (26.0)	40 (39.2)	46 (45.1)	16 (15.9)
51 - 60	51 (13.0)	20 (39.2)	29 (56.9)	2 (3.9)
>60	48 (12.2)	18 (37.5)	23 (47.9)	7 (14.6)
Total	**393 (100)**	**158 (40.2)**	**188 (47.8)**	**47 (12.0)**

* Numbers in parenthesis are percentages.

It was also found that 46.9% (23/49) of those with no formal education rated laboratory diagnosis as not important. Others were 45.3% (82/181) of caregivers with secondary education, 33.1% (42/127) among caregivers with primary education and least 30.6% (11/36) was in those with tertiary education. 53.5% (68/127) of caregivers with primary education rated laboratory diagnosis as barely important. Others were 46.9% (23/49) of those with no formal education, 45.3% (82/181) of those with secondary education and the least 41.7% (15/36) among those with tertiary education. Amidst those that rated malaria laboratory diagnosis

very important, 27.8% (10/36) were among those with tertiary education. Others were 13.4% (17/127) of those with primary education, 9.4% (17/181) of those with secondary education and the least 6.1% (3/49) of those with no formal education as shown in table 9. The observed differences were not statistically significance ($P > 0.05$).

Table 9: Caregivers assessment of the importance of laboratory diagnosis according to educational status.

Educational status	No. sampled (%)	Degree of importance of laboratory diagnosis of malaria (% response)		
		Not important (%)	Barely important (%)	Very important (%)
Informal	49 (12.5)	23 (46.9)	23 (46.9)	3 (6.1)
Primary	127 (32.3)	42 (33.1)	68 (53.5)	17 (13.4)
Secondary	181 (46)	82 (45.3)	82 (45.3)	17 (9.4)
Tertiary	36 (9.2)	11 (30.6)	15 (41.7)	10 (27.8)
Total	**393 (100)**	**158 (40.2)**	**188 (47.8)**	**47 (12.0)**

* Numbers in parenthesis are percentages.

On the mode of communication of information on laboratory diagnosis suggested by caregivers, 38.7% (152/393) of the caregivers suggested use of community health workers (CHWs). Other modes suggested were radio/TV by 21.4% (84/393), church by 16.8% (66/393), community meetings (CM) by 13.0% (51/393) and the least suggested was pamphlets by 10.2% (40/393) of the caregivers. Among caregivers aged 21-30 years, 37.8% (28/74) suggested radio/TV, 36.5% (27/74) pamphlets, 16.2% (12/74) CHWs,

9.5% (7/74) church and none for CM. For caregivers in age bracket 31-40 years, highest suggestion of 56.8% (67/118) was made on CHWs. Others were church 18.6% (22/118), radio/TV 14.4% (17/118), CM 8.5% (10/118) and the least was pamphlets 1.7% (2/118). 39.2% (40/102) in age bracket 41-50 years suggested CHWs, 19.6% (20/102) radio/TV, 18.6% (19/102) CM, 17.6% (18/102) church, and least was pamphlets 4.9% (5/102). Amid those aged 51-60 years, 29.4% (15/51) suggested CHWs, 23.5% (12/51) CM, 21.6% (11/51) radio/TV, 17.6% (9/51) church and the least was pamphlets 7.8% (4/51). Among > 60 years, 37.5% (18/48) suggested CHWs, 16.7% (8/48) radio/TV, 20.8% (10/48) each for church and CM, and 4.2% (2/48) for pamphlets as shown in table 10. The observed difference was not statistically significance (P > 0.05).

Table 10: Mode of communication for enlightenment on malaria diagnosis suggested by caregivers in Ozubulu according to age.

Age in years	No. sampled (%)	Mode of communication suggested by caregivers (% response)				
		Church (%)	Radio/TV (%)	Pamphlets (%)	Community health workers (%)	Community meetings (%)
21 – 30	74 (18.8)	7 (9.5)	28 (37.8)	27 (36.5)	12 (16.2)	0 (0.0)
31 – 40	118 (30.0)	22 (18.6)	17 (14.4)	2 (1.7)	67 (56.8)	10 (8.5)
41 – 50	102 (26.0)	18 (17.6)	20 (19.6)	5 (4.9)	40 (39.2)	19 (18.6)
51 - 60	51 (13.0)	9 (17.6)	11 (21.6)	4 (7.8)	15 (29.4)	12 (23.5)
>60	48 (12.2)	10 (20.8)	8 (16.7)	2 (4.2)	18 (37.5)	10 (20.8)
Total	**393 (100)**	**66 (16.8)**	**84 (21.4)**	**40 (10.2)**	**152 (38.7)**	**51 (13.0)**

* Numbers in parenthesis are percentages.

It was also found that among those with no formal education, 44.9% (22/49) suggested CHWs, 42.9% (21/49) CM, 12.2% (6/41) church and none suggested either radio/TV or pamphlets. For caregivers that attended just primary education, 40.2% (51/127) suggested CHWs, 23.6% (30/127) CM and church each, 12.6% (16/127) radio/TV and none suggested pamphlets. Amongst those with secondary education, 33.7% (61/181) suggested CHWs, 32.6% (59/181) radio/TV, 17.1% (31/181) pamphlets, 16.6% (30/181) church, and none suggested CM. Lastly, amid caregivers with tertiary education, 50% (18/36) suggested CHWs, 25% (12/51) suggested radio/TV and pamphlets each, and none opt for church or CM. The observed difference was not statistically significance (P > 0.05).

Table 11: Mode of communication for enlightenment on malaria diagnosis suggested by caregivers in Ozubulu according to educational status.

Educational status	No. sampled (%)	Mode of communication suggested by caregivers (% response)				
		Church (%)	Radio/TV (%)	Pamphlets (%)	Community health workers (%)	Community meetings (%)
Informal	49 (12.5)	6 (12.2)	0 (0.0)	0 (0.0)	22 (44.9)	21 (42.9)
Primary	127 (32.3)	30 (23.6)	16 (12.6)	0 (0.0)	51 (40.2)	30 (23.6)
Secondary	181 (46)	30 (16.6)	59 (32.6)	31 (17.1)	61 (33.7)	0 (0.0)
Tertiary	36 (9.2)	0 (0.0)	9 (25.0)	9 (25.0)	18 (50.0)	0 (0.0)
Total	**393 (100)**	**66 (16.8)**	**84 (21.4)**	**40 (10.2)**	**152 (38.7)**	**51 (13.0)**

* Numbers in parenthesis are percentages.

CHAPTER FIVE

DISCUSSION

Pregnancies in women living in malaria endemic regions, particularly in sub–Saharan Africa are associated with a high frequency and density of *Plasmodium falciparum* parasitaemia, with high rates of maternal morbidity (Mkandala, 2003). *P. falciparum* being the only species found in this study is in line with studies of Abdullahi *et al.*, 2009 and Adefioye *et al.,* 2007 which showed that *P. falciparum* is the most dominant species in pregnancy. In highly endemic malarious area where semi-immune adults usually have substantially acquired resistance to local strains of *Plasmodia*, the prevalence of clinical malaria is higher and its severity greater in pregnant women than in non-pregnant women (Okwa, 2003). High prevalence rate of 53.9% of malaria parasite recorded in this was in accordance with 64.4% and 63.6% prevalence reported by Aribodor *et al.*, 2009 and Akinboro *et al*., 2010 respectively. Though the prevalence of this study was rather lower than these two studies mentioned above, this may be attributed to the improved understanding of the Antenatal Clinic women about malaria control strategies like use of long lasting insecticide treated nets (LLIN) or alternative intermittent preventive treatment with pyrimethamine-sulfadoxine (SP). The lower prevalence might also be for the reason that this study was conducted during the dry season. According to Ayanda 2009, prevalence of *P. falciparum* infection is higher in the wet season than in the dry season. Minakaw *et al*., 2002 opined

that the rainy season presents favourable environmental conditions that enhance mosquito breeding and survival, through the proliferation of larval habitats and improved humidity respectively.

In studies conducted by Brabin in 1991 and Onwere *et al*., 2008, the primigravidae were more susceptible to malaria infection than the multigravidae, as confirmed by this study. Prevalence was highest among the primigravidae (65.8%) and malaria positivity decreased as parity increased. Among the secundigravidae, prevalence was found to be 61.1%, while Para 3, and 4 and above had prevalence of 51.2% and 43.0% respectively. Mcgregor 1984, identified the factors responsible for susceptibility of primigravidae to malaria as inhibition of type 1 cytokine responses (interferon, interleukins 2 and 12 and TNF). Cell-mediated immune responses to malaria antigens are more markedly suppressed in first than in subsequent pregnancies (Brabin, 1996). The multigravidae are presumably less affected because immunological memory from first pregnancy is retained (Brabin, 1996).

Younger women appeared to be susceptible to malaria in this study as prevalence was highest among age group 21 - 25 (68.8%). This contradicted the findings of Adefioye *et al.,* 2007 that found 36 - 39 year old group to be more susceptible, but agreed with the findings of Dicko *et al.,* (2003) who opined that adolescents and young adult pregnant women were more susceptible to malaria than older pregnant women, because of continuous development of malaria immunity in older women.

First trimester prevalence in this study is in line with previous studies as Brabin in 2000 found in western Kenya that prevalence was highest at 13 – 16 weeks gestation (1st trimester), and found similar number of recoveries in both groups during the 2nd and 3rd trimesters. The loss of immunity in early pregnancy was equivalent to an 11-fold decrease in the rate of recovery from infection (Brabin, 2000). The recovery seen in the late pregnancy suggests that the women mount a satisfactory immune response to malaria infection, re-acquiring their pre-pregnancy immune status at about the time of delivery (Saute *et al*., 2002). The observation could also be as a result of constant intermittent preventive treatments in pregnancy (IPTp) given to pregnant women during antenatal care visit which usually commence during second trimester.

Community knowledge and attitudes to diagnosis demonstrate the importance of providing patients with a reliable explanation for their illness. This improves treatment-seeking behaviour and compliance. It was impressive to discover that knowledge about signs and symptoms of malaria is relatively high in this study with most respondents indicating awareness of key symptoms including fever, headache, chills, weakness, and joint/body pains. This is in line with the observations of most studies in endemic settings (Adedotun *et al.*, 2010; Hlongwana *et al*., 2009; Oreagba *et al*., 2004). Federal Ministry of Health (FMOH) survey assumed that the households had good knowledge of the symptoms of malaria if they mentioned at least fever plus headache or other pain but poor knowledge if they mentioned fever plus general weaknesses or

dizziness. In this study, majority of the caregivers had good knowledge of the symptoms of malaria. This was also seen in study of Oreagba *et al*., (2004) at urban Ado-Odo in Ogun, where majority of households were considered to have good knowledge of the symptoms of malaria. Nevertheless according to Bell *et al.,* 2001, observation of fever alone, and of fever in combination with chills and/or headache, achieved quite high sensitivities, but both criteria resulted in high rates of overtreatment and, any narrower combination of symptoms resulted in sensitivities unacceptable in relation to the detection of a life-threatening illness.

Malaria Rapid Diagnostic Tests (RDTs) have been recommended to improve diagnostic efficiency, which is important for preventing indiscriminate use of Artemisinin-Based Combination Therapy (ACT), thereby preventing or delaying the development of parasite resistance to this new first-line drug (Msellem *et al.,* 2009). RDTs can be used as a stop-gap when microscopy services are not operating (e.g. evenings/weekends/public holidays) or as a primary diagnostic tool for rural/remote areas without microscopy services (MOH and MS, 2009). Disappointedly, survey on knowledge of caregivers in this study on malaria diagnostic methods showed that only a few participants (2.5%) in the community reported knowing what RDTs are or had experienced this test. This was also seen in work of Rushika *et al*., 2011 where none of the participants in the urban village and very few from the rural village knew what RDTs were.

Their opinion on malaria laboratory diagnosis showed poor mind-set towards the importance. This poor attitude to malaria confirmatory diagnosis could possibly be as a result of their poor knowledge of its importance. Most participants recommended for further awareness to be carried out in order to improve community knowledge on importance of laboratory diagnosis. Many of the participants suggested that this awareness will most effectively be carried out by community healthcare workers or trained volunteers through regular visits to communities, further stating that it would allow villagers to receive education, ask questions and have their concerns addressed. Some suggested distribution of pamphlets containing basic information on laboratory diagnosis, while others recommended educating people through radio/TV.

CONCLUSION

This study recorded high prevalence of malaria parasitaemia among pregnant women attending antenatal clinic in Ozubulu. The high prevalence of malaria parasites once more brings to fore the endemicity of malaria in Anambra State, which is in the savannah belt of south-eastern Nigeria, thus a good environment for mosquito breeding, especially in the rainy season. Though this study was conducted in the dry season, poor environmental sanitation favoured the breeding of mosquitoes in the study community.

Regular environmental sanitation to dislodge mosquitoes from their breeding places will go a long way to reduce prevalence of malaria in villages commonly seen in the tropics. Early antenatal booking

for effective monitoring and prompt treatment of malaria in pregnancy will contribute significantly in reducing maternal morbidity and mortality, and its perinatal mortality, and it is of necessity that routine intermittent preventive treatment of malaria is recommended for pregnant women in this area. Several studies have shown that protection against malaria contributes to the prevention of malaria in pregnancy, thus highlighting the importance and efficacy of chemoprophylaxis and use of other methods of malaria control like insecticide impregnated nets.

Therefore, it is necessary to enlighten them on importance of intermittent preventive treatment of malaria and use of insecticide treated nets in pregnancy. Educating them on the importance will augment the acceptance of and adherence to these new preventive measures, which are crucial to improving the effectiveness of malaria preventive control, by limiting placental malaria and its adverse effects on foetal outcomes; and improving child survival and development, thus reducing the burden of malaria in endemic areas.

Practice of presumptive treatment is of considerable concern for both the health of individuals and the increased risk it poses to the development of parasite resistance to the first-line treatment against malaria (WHO, 2010). Malaria RDTs have been recommended to improve diagnostic efficiency. These RDTs have opened new possibilities for improved rural malaria diagnosis that is independent of centralized diagnostic services (Bojang, 1999;

Singh *et al*., 1997). But considering the poor knowledge of community members to RDTs and poor attitude to laboratory importance, it is of great necessity that intensive health education should be implemented to inform communities about the new treatment regime, as well as effective refresher training of healthcare workers, with follow-on activities such as job aides and supportive supervision on the use of RDTs.

In addition, supply lines should be streamlined for RDTs to ensure consistent stocks in the healthcare centres. Considering the high level of poverty in Nigeria, with 63% living on below $1 per day (Daily Sun, 2011), government should create an avenue of subsidy in health services, both in diagnosis and treatment of malaria. These recommendations will be integral to the success and sustainability of intensified malaria control, subsequently leading to elimination of malaria in Nigeria.

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APPENDIX I

QUESTIONNAIRE

Introduction: I am Obianumba, Stella Nnenna, an M. Sc student of the Department of Parasitology and Entomology, Nnamdi Azikiwe University, Awka. I am conducting a research on knowledge and attitude of community members to diagnosis of malaria in Ozubulu, Ekwusigo Local Government Area, Anambra State.

Purpose of the research: The present research will help in malaria control program to better understand the informational needs of the community on malaria issues.

Procedure: If you agree with the purpose of the research, I will question you about your knowledge and attitude in relation to malaria diagnosis. The questioning will last approximately 15 minutes.

Benefits: There are no direct benefits for you being part of this research. However, your contribution will help immensely to a better malaria control program in the community. You are free not to participate in this research or not to answer any question you feel uncomfortable with. Should you agree to be part of the research, feel free to interrupt the interview process at any time. Confidentiality is guaranteed and your answer will be part of many household interviews so that anonymity is ensured. Your name will not appear in any oral or written report of this study. There are no wrong or right answers. Your openness and honest opinions are extremely important. In case you do not understand a question or issue, please ask me to repeat or clarify.

Would you like to participate in this study? Yes ( ) No ( )

Section 1

Demographical data

1. Age in years 21-30 ( ) 31-40 ( ) 41-50 ( ) 51-60 ( ) > 60 ( )

2. Marital status Single ( ) Married ( ) Divorced ( ) Widowed ( )

3. Occupation Traders ( ) Farmers ( ) Civil servants ( )
Unskilled labourers ( ) Pensioners ( )

4. Level of Education informal ( ) Primary ( ) Secondary ( ) Tertiary ( )

Section 2

5. Have you heard about malaria? Yes ( ) No ( )

6. Which are the common signs and symptoms of malaria?
Headache ( ) Fever ( ) Chill and shivering ( ) Body pains ( ) Loss of appetite ( ) Vomiting ( ) Weakness ( ) Dizziness ( ) Don't know ( )

7. Have you heard about malaria diagnosis? Yes ( ) No ( )

8. Which of these malaria diagnoses do you know about?
Self diagnosis ( ) Microscopy ( ) Rapid Diagnostic Test ( ) Others specify.....

9. How important will you rate going for laboratory test before treatment of malaria? Very important ( ) Barely important ( ) Not important ( )

10. Where would you like information on malaria diagnosis communicated to the community members? (*Through what channels of communication?*)
Church ( ) Radio/TV ( ) Pamphlets ( ) Community Health Workers ( )
Community meetings ( ) Others specify .....

We have concluded our interview. Thank you very much for your hospitality and valuable contribution. Do you have any question for me?

APPENDIX II

Sample Size Calculations

$$n = \frac{N}{1 + N(e^2)}$$

n = sample size
N = total population
e = error term at 95% confidence interval

Sample size for pregnant women in Ozubulu

Female population in 2006 = 21,828

Female of reproductive age = 49% of female population

Population of pregnant women = 5% of female of reproductive age

Population growth = 3%

$$\text{Female population in 2007} = \frac{(3 \times 21{,}828) + 21{,}828}{100} = 22{,}483$$

$$\text{Female population in 2008} = \frac{(3 \times 22{,}483) + 22{,}483}{100} = 23{,}157$$

$$\text{Female population in 2009} = \frac{(3 \times 23{,}157) + 23{,}157}{100} = 23{,}852$$

$$\text{Female population in 2010} = \frac{(3 \times 23{,}852) + 23{,}852}{100} = 24{,}568$$

$$\text{Female population in 2011} = \frac{(3 \times 24{,}568) + 24{,}568}{100} = 25{,}305$$

$$\text{Female of reproductive age} = \frac{49 \times 25{,}305}{100} = 12{,}399.45 \approx 12{,}399$$

$$\text{Population of pregnant women} = \frac{5 \times 12{,}399}{100} = 619.95 \approx 620$$

Sample size for pregnant women = $\frac{620}{1+(620\times0.05^2)}$ = 243.14 ≈ 243

Sample size for caregivers in Ozubulu

Total population in 2006 = 46,062

Adult population = 45% of total population

Population growth = 3%

Total population in 2007 = $\frac{(3 \times 46{,}062) + 46{,}062}{100}$ = 47,444

Total population in 2008 = $\frac{(3 \times 47{,}444) + 47{,}444}{100}$ = 48,867

Total population in 2009 = $\frac{(3 \times 48{,}867) + 48{,}867}{100}$ = 50,333

Total population in 2010 = $\frac{(3 \times 50{,}333) + 50{,}333}{100}$ = 51,843

Total population in 2011 = $\frac{(3 \times 51{,}843) + 51{,}843}{100}$ = 53,398

Adult population = $\frac{49 \times 53{,}398}{100}$ = 24,029

Sample size for caregivers = $\frac{24{,}029}{1+(24{,}029\times0.05^2)}$ = 393

APPENDIX III

Crosstab and Pearson Chi-square to establish possible correlation and test significance of prevalence with parity, age, trimester

Parity * Prevalence of malaria Cross tabulation

Count

Parity		Prevalence of malaria		Total
		positive	negative	
Number of pregnancy	primigravid	48	25	73
	secundigravid	22	14	36
	multigravid - 3	21	20	41
	multigravid -4 & above	40	53	93
Total		**131**	**112**	**243**

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	9.438[a]	3	.024
Likelihood Ratio	9.525	3	.023
Linear-by-Linear Association	9.300	1	.002
N of Valid Cases	243		

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 16.59.

Gestation period * Prevalence of malaria Cross tabulation

Count

Gestational age		Prevalence of malaria		Total
		positive	negative	
Gestation period	first trimester	58	23	81
	second trimester	31	36	67
	third trimester	42	53	95
Total		**131**	**112**	**243**

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	15.379[a]	2	.000
Likelihood Ratio	15.796	2	.000
Linear-by-Linear Association	12.685	1	.000
N of Valid Cases	243		

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 30.88.

Age of the pregnant women * Prevalence of malaria Cross tabulation

Count

Age in years		Prevalence of malaria		Total
		Positive	negative	
Age of the pregnant women	16-20	24	12	36
	21-25	33	15	48
	26-30	42	24	66
	31-35	18	26	44
	36-40	11	23	34
	41-45	3	12	15
Total		**131**	**112**	**243**

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	25.419[a]	5	.000
Likelihood Ratio	26.053	5	.000
Linear-by-Linear Association	21.572	1	.000
N of Valid Cases	243		

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 6.91.

APPENDIX IV

Crosstab and Chi-Square Tests on Knowledge of Signs and Symptoms of Malaria according to Demographical Data

Age of the caregivers * common signs and symptoms of malaria Cross tabulation

Count

Age in years	Common signs and symptoms of malaria						No. sampled
	Chill	Fever	Headache	Joints /body pain	Weakness	Others	
21 – 30	60	74	51	21	21	27	74
31 – 40	94	118	83	62	30	36	118
41 – 50	92	102	86	60	32	38	102
51 - 60	48	51	45	34	18	16	51
>60	46	48	41	38	16	14	48
Total	**340**	**393**	**306**	**215**	**117**	**131**	**393**

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	50.840[a]	4	.271
Likelihood Ratio	65.134	4	.016
Linear-by-Linear Association	1.036	1	.309
N of Valid Cases	393		

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 7.82.

Educational status of the caregivers * common signs and symptoms of malaria Cross tabulation

Count

Educational status	Common signs and symptoms of malaria						Total No. Sampled
	Chill	Fever	Headache	Joints /body pain	Weakness	Others	
Informal	36	49	30	21	19	18	49
Primary	111	127	103	60	41	48	127
Secondary	163	181	139	106	46	59	181
Tertiary	30	36	34	28	11	6	36
Total	**340**	**393**	**306**	**215**	**117**	**131**	**393**

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	103.594[a]	3	.133
Likelihood Ratio	110.315	3	.011
Linear-by-Linear Association	92.504	1	.013
N of Valid Cases	393		

a. 0 cell (.0%) have expected count less than 5. The minimum expected count is 5.86.

Appendix V

Crosstab and Chi-Square Tests on Knowledge of Different Malaria Diagnostic Methods according to Demographical Data

Age of the caregivers * knowledge of malaria diagnostic methods Cross tabulation

Count

Age in years	Knowledge of malaria diagnostic methods			No. sampled
	Self diagnosis	Microscopy	RDT	
21 – 30	74	31	2	74
31 – 40	118	67	3	118
41 – 50	102	71	2	102
51 - 60	51	24	0	51
>60	48	31	3	48
Total	**393**	**224**	**10**	**393**

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	61.037[a]	4	.231
Likelihood Ratio	66.392	4	.000
Linear-by-Linear Association	16.299	1	.037
N of Valid Cases	393		

a. 1 cell (10.0%) has expected count less than 5. The minimum expected count is 3.26.

Educational status of the caregivers * knowledge of malaria diagnostic methods Cross tabulation

Count

Educational status	Knowledge of malaria diagnostic methods			No. sampled
	Self diagnosis	Microscopy	RDT	
Informal	49	5	0	49
Primary	127	58	0	127
Secondary	181	125	0	181
Tertiary	36	36	10	36
Total	**393**	**224**	**10**	**393**

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	7.221[a]	3	.013
Likelihood Ratio	8.145	3	.019
Linear-by-Linear Association	.256	1	.018
N of Valid Cases	393		

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 5.74.

Appendix VI

Crosstab and Chi-Square Tests on Degree of Importance of Laboratory Diagnosis of Malaria according to Demographical Data

Age of the caregivers * Degree of importance of laboratory diagnosis of malaria Cross tabulation

Count

Age in years	Degree of importance of laboratory diagnosis of malaria			No. sampled
	Not important	Barely important	Very important	
21 – 30	30	38	6	74
31 – 40	50	52	16	118
41 – 50	40	46	16	102
51 - 60	20	29	2	51
>60	18	23	7	48
Total	**158**	**188**	**47**	**393**

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	10.378[a]	4	.116
Likelihood Ratio	10.320	4	.012
Linear-by-Linear Association	.331	1	.565
N of Valid Cases	393		

a. 1 cells (12.5%) have expected count less than 5. The minimum expected count is 2.28.

Educational status of the caregivers * Degree of importance of laboratory diagnosis of malaria Cross tabulation

Count

Educational status	Degree of importance of laboratory diagnosis of malaria			No. sampled
	Not important	Barely important	Very important	
Informal	23	23	3	49
Primary	42	68	17	127
Secondary	82	82	17	181
Tertiary	11	15	10	36
Total	**158**	**188**	**47**	**393**

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	48.043[a]	4	.134
Likelihood Ratio	46.402	4	.016
Linear-by-Linear Association	15.312	1	.182
N of Valid Cases	393		

a. 1 cell (10.0%) have expected count less than 5. The minimum expected count is 3.26.

Appendix VII

Crosstab and Chi-Square Tests on Degree of Importance of Laboratory Diagnosis of Malaria according to Demographical Data

Age of the caregivers * Degree of importance of laboratory diagnosis of malaria Cross tabulation

Count

Age in years	Mode of communication suggested by caregivers					No. sampled
	Church	Radio/TV	Pamphlets	Community health workers	Community meetings	
21 – 30	7	28	27	12	0	74
31 – 40	22	17	2	67	10	118
41 – 50	18	20	5	40	19	102
51 - 60	9	11	4	15	12	51
>60	10	8	2	18	10	48
Total	**66**	**84**	**40**	**152**	**51**	**393**

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	33.063[a]	4	.281
Likelihood Ratio	13.421	4	.052
Linear-by-Linear Association	10.308	1	.182
N of Valid Cases	393		

a. 1 cell (10.0%) have expected count less than 5. The minimum expected count is 3.26.

Educational status of the caregivers * Degree of importance of laboratory diagnosis of malaria Cross tabulation

Count

Educational status	Mode of communication suggested by caregivers					No. sampled
	Church	Radio/TV	Pamphlets	Community health workers	Community meetings	
Informal	6	0	0	22	21	49
Primary	30	16	0	51	30	127
Secondary	30	59	31	61	0	181
Tertiary	0	9	9	18	0	36
Total	**66**	**84**	**40**	**152**	**51**	**393**

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	9.903[a]	3	.183
Likelihood Ratio	6.353	3	.201
Linear-by-Linear Association	4.472	1	.113
N of Valid Cases	393		

a. 1 cell (10.0%) have expected count less than 5. The minimum expected count is 3.26.