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The Assessment of the Efficacy of the Usage of LLIN among Pregnant Women in Ile-Ife, Osun State.

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Abstract: *Malaria remains one of the leading causes of maternal morbidity and mortality despite being preventable and treatable. The infection put both the mother and the unborn child at risks such as maternal anaemia, low birth weight, abortion and still birth. Although, enormous efforts had been embarked upon such as, the use of ITNs/LLINs, IPTp-SP . Hence, the study was designed to assess the incidence of malaria and the efficacy of LLINs among child bearing women attending Tofemedics Diagnostic Centre, Ile-Ife, Osun State. Two hundred and fifty pregnant women and fifty non-pregnant women were examined. Blood microscopy was done for the presence of malaria parasite and estmation of packed cell volume. And the use of preventive measure against mosquito bite was obtained through structured questionnaire. Seventy percent (70%) of the pregnant women were infectedcompared to non-pregnant women. 48.8% of the infected pregnant women are primigravidae and the women in their first trimester are the most affected based on gestational age.Out of one hundred and eight pregnant women who possess LLIN, only 44% of are sleeping under it. The usage of ITNs/LLINs among the two groups of women is statistically not significant (P> 0.005). None of the pregnant women had severe anaemia but one of the non-pregnant women had severe anaemia. The study elucidate that malaria burden is still enormous among the pregnant women attending private diagnostic centre in Ile-Ife, Osun State despite all the control strategy measure put in place by the Government.*

Keywords:*Malaria, ITNs, LLIN, Pregnant Women, Ile-Ife*

I. Introduction

Malaria is a disease caused by parasitic infection of*Plasmodium* species.Although preventable and treatable, malaria takes number one cause of human morbidity and mortality among the six leading parasitic diseases.The parasite was first discovered by Charles Louis Laveran, a French army surgeon stationed in constantine,Algeria, in the blood of a patient suffering from malaria on the 6th November, 1880 (CDC, 2010; Cox, 2010).According to the World Health Organization (2014), malaria cases worldwide were about 198 million, with 584 thousand deaths in 2013. Globally, malaria caused an estimated 453 thousand deaths among the children less than 5 years old in 2013 and almost 3% of Disability Adjusted Life Years (DALYs) are due to malaria mortality (Breman *et al.,* 2004; WHO, 2014). Malaria is a complex disease that varies widely in epidemiology and clinical manifestations in different parts of the world. Variable factors such as distribution and efficiency of mosquito vector, climate and other environmental conditions, as well as behavior and level of acquired immunity of the exposed population contributes to wide distribution of malaria (CDC, 2001). Likewise, severity and life threatening of malaria has been thought to be determined by the interaction of a number of factors. These include the size of the infective dose of sporozoites, nutritional status of the host, level of acquired immunity, host genetic factors such as the presence of sickle cell hemoglobin, parasite features such as growth rate and drug resistance, and socioeconomic factors as basic as the availability of health care and education (Philips, 2001).In Nigeria, malaria is a major public health disease in which 97% is at the risk of the infection. It accounts for 30% and 11% childhood and maternal mortality respectively. It also accounts for more than 60% of outpatient visit to the hospital (FMoH, 2005). While 50% of the adult population in Nigeria experience at least one episode of malaria annually, children under 5yrs of age experience 2 - 4 bouts (FMoH, 2005). Malaria in pregnancy is mostly of high morbidity and mortality in sub-Saharan Africa. Pregnant women are more susceptible to *Plasmodium* infection and are more symptomatic when compare with non-pregnant women. Lindsay*et al.* (2000) observed that the pregnant women are more attracted to mosquito which transmits the disease. Likewise, Kakkilaya (2011) was of the opinion that hormonal changes of pregnancy, reduced sythesis of immunoglobulins, and reduced function of reticulo-endothelial system are the cause for immunosuppression in pregnancy. This result in loss of acquired immunity to malaria, making the pregnant

women more prone to malaria. Moreover, during pregnancy all pregnant women retain water in their blood vesselsand do not make enough red blood cells to compensate (Takem and D' Alessandro,2013).
Infection with the most virulent of the four human species, *Plasmodium falciparum*, mostly results in maternal illness and low birth weight. The symptoms and complications of malaria in pregnancy vary according to malaria transmission intensity in the given geographical area, and the individual's level of acquired immunity (WHO, 2015). The parasite may not be found in the peripheral blood but sequestered in the organs especially the placental. The presence of parasite in the placental contributes to maternal anaemia and placental malaria. Both can lead to low birth weight which greatly contributes to infant mortality.

Women experiencing malaria in pregnancy may exhibit some normal symptoms of malaria such as high fever, severe chills with shaking followed by episodes of fever and sweating, body aches, anaemia, mild jaundice, enlarge spleen and fall in the body temperature. But some may be asymptomatic or present with mild symptoms such as lack of the characteristics fever which may prevent a woman from seeking treatment despite the danger to herself and her unborn child (Desa *et al*., 2011; CDC 2011;Health plus 24 team, 2013). In high-transmission settings, the adverse effects of *Plasmodium falciparum* infection are most pronounced in primigravidae (WHO, 2015).

It had been observed that about 125million pregnancies are at risk of malaria every year and up to 200,000 babies die as a result, with at least 10,000 maternal deaths per annum in sub Sahara Africa (CDC, 2010). In high-transmission areas it causes maternal anaemia, higher rates of miscarriages, intrauterine demise, premature delivery, still birth, low birth weight and neonatal death; while in low-transmission areas and in non-immune individuals, malaria in pregnancy is associated with development of severe disease (Bell and Winstanley, 2004; Hartman *et al*., 2010; Seal *et al.*, 2010; Rijken *et al*., 2012).

Prevention of malaria disease in pregnancy relies on three main strategies as recommended by the World Health Organization; the use of insecticides treated bed nets (ITNs/LLINs), Intermittent Presumptive Treatment (IPTp) with Sulfadoxine-Pyrimethamine (SP) and prompt and effective treatment of malaria cases. This is refers to as administration of 2 or more doses of chemoprophylaxis after 20 weeks of gestation to reduce the malaria parasite load (Kiwuwa and Mufubenga, 2008). However, only few (about 25%) of pregnant women at risk of malaria infection receive at least one dose of IPTp-SP (Clara, 2015).

Moreover, Nigeria Government under the integrated vector management scheme, put in place the distribution of Long Lasting Insecticide Nets (LLINs) to pregnant women attending first ante natal care (ANC) (Okorie, 2011). Despite this, quite a number of pregnant women are yet to be free from the scourge of malaria in pregnancy. This geared the study on malaria in pregnancy to survey pregnant women infected with Plasmodium and the efficacy of LLINs in the control of malaria in pregnancy.

II. Materials and Methods

2.1 Study Area

The study was carried out at Tofemedics Diagnostic Centre, Ile-Ife Osun State.Ile-Ifeis a town in Osun state, Southwestern Nigeria. The town lies at the intersection of roads from Ibadan (40 miles [64 km] west),Ilesa and Ondo. It is one of the largestcentres and probably the oldest town of the Yoruba people. The climate of the area is typically tropical with a characteristic dry season of about 6 months (October- March) and a wet season of about 6 months (April- September) (Akinbuwa and Adeniyi, 1996). The mean annual rainfall ranges between 1000 and 1250m (Oguntoyinbo, 1982), the mean annual relative humidity ranges from 75% to 100%(Ayoade, 1982), and the mean annual temperature is about 30˚C (Ndifon and Ukoli, 1989). The vegetation of the area is tropical rain forest characterized by large and tall trees.The inhabitants of the area are mixture of people from different ethnic groups in Nigeria. However, the majority are the Yoruba speaking people of the south-west.

2.2. Study Subjects

The approval for the study was obtained from the management of Tofemedics Diagnostic Centre. Both pregnant and non-pregnant women that present themselves at the centre were recruited for the study. However, only those that gave their assent after the concept of the study had been explained verbally, while some through written were used for the study. Each of the subjects was made to sign an agreement form to validate their willingness to participate in the study. Three hundred women were recruited for the study. Two hundred and fifty were pregnant women while the remaining fifty non-pregnant women served as control group.

2.3. Sample Collection

Blood samples were obtained from the peripheral blood of the women from January- June, 2015 using sterile lancet needles. A needle each was used for individual participant. Heparinized capillary tube was use to collect peripheral blood for the determination of packed cell volume.

Questionaires were used to get some parameters such as; gestation age, number of pregnancy (primi/multigravid), use of bed net or window net, use of insecticide. Likewise, environmental conditions such as presence of stagnant water were recorded from the entire subject involved.

2.4.Processing of Samples

2.4.1 Blood Films

Blood samples were obtained from the peripheral blood of the study subjects using finger prick. The third finger was swab with 70% alcohol and allowed to dry before pricking. Thick and thin films were made on clean, well-labelled grease-free slide. One drop for thin film and three drops for thick film about 15 mm to the right in a triangular way on the same glass slide.Two slides were made for each study participant. A small drop of absolute methanol was applied to the thin film to fix for 1–2 minutes, making sure the alcohol does not touch the thick film as this will prevent lyses of the red cells and make the thick film unreadable, the slide was placed in slant position to air dry (WHO, 2001).

The blood films were stained with 10% Giemsa stain for 10 minutes. The Giemsa stain was prepared with buffered distilled water at pH 7.2. The films were allowed to air dried (Cheebrough, 2005). The stained slides were read by two microscopists using x 100 objective lens. One hundred fields were checked before declaring a slide negative.

2.4.2 Estimation of Packed Cell Volume

Peripheral Blood was collected using finger prick. The finger was cleanse with 70% ethanol, a sterile lancet was used to prick the finger, the first drop of blood was wiped away with a dry piece of cotton wool and was hold tightly to obtain a large drop of blood into the heparinized capillary tube which is moved closely to the finger to collect the blood. The tube was wiped with cotton and the unmarked end was sealed with plasticine, the number of patient's sample was written against the number on the centrifuge. Two tubes were collected from each subject.One of the two tubes was placed in the haematocrit centrifuge and spun for five minutes at 12,000rmp speed (Cheesbrough, 2005). After centrifuge for 5mins, the hyparinized tubes were checked if there has been leakage of blood from the capillary or breakage. Where there is leakage or breakage, the reserved tube was used.The PVC was read using microhaematocrit reader.

III. Results

Two hundred and fifty pregnant women and fifty non pregnant women were recruited for the study. Two hundred and ten (84%) pregnant women were infected with *Plasmodium* while forty (16%) were not infected (Fig 1). Table 1 explains the prevalence of infection among both pregnant and non-pregnant women. Twenty two (7%) of non pregnant women were *Plasmodium* positive and twenty eight (9%) were negative. There was no relationship in the *Plasmodium* infection rate among the two groups($P>0.01$, $df=2$).

Out of the total pregnant women examined in the study, one hundred and forty four were primigravidae while one hundred and six were multigravidae. One hundred and twenty-one (85%) of the primigavidae were parasitaemic while twenty three (15%) were not infected. Eighty-nine (84%) of the multigravidae were parasitaemic while sixteen were not infected (Table 2).

Among the two hundred and ten *Plasmodium*infected pregnant women, fifty two (25%) were anemic while 158 (75%) were non anemic. Thirteen (33%) of *Plasmodium* non - infected pregnant women were anemic while twenty-seven (67%) were non anemic. There was no association between *Plasmodium*infection and anemicity ($P>0.05$). Out of the fifty non- pregnant women, twenty two of them were parasitemic while twenty eight were not parasitemic. Among the twenty two that were parasitemic, 4 and 18 were anemic and non anemic respectively, while 8 and 20 among *Plasmodium*non-infected were anemic and non- anemic respectively. The prevalence of anemicity among the pregnant women and non pregnant women was statistically significant ($P<0.005$)(Table 3).

On the awareness of prophylactics, seventy eight (31.2%) of the pregnant women had the knowledge of IPTp during pregnancy but only fifty one women (65% of 78) were given. For the preventive measure against malaria applied by the women,One hundred and eight of the pregnant women possess LLIN, out of which only Forty eight (44%) use the net while sixty (56%) were not using. Out of 50 non pregnant women recruited for the study, thirty five possess LLIN but only sixteen (46%) use the net (Fig. 2). The attitude of both pregnant and non pregnant women towards the use of LLIN was statistically less significant ($P>0.005$).

Among the Forty-eight pregnant women using LLIN, forty two had malaria parasite in their blood while six were negative. Fifty two among non-LLIN users also had malaria parasite in their blood and eight were negative. Six among the non pregnant LLIN users had malaria parasite in their blood and ten were negative while nine among the non pregnant and non-LLIN users were *Plasmodium*positive and ten were negative. There was no statistical significance (*$P>0.01$*) in the relationship between LLIN usage and *Plasmodium*infection. However, 75% (48 out of 64) had infection despite the use of LLIN (Table 4).

Twenty- nine pregnant women among LLIN users go to bed between 9pm and 10pm while twenty (20) and three (3) go to bed latest 8pm and 12 midnight respectively (Fig 3). Figure 4 shows the factors preventing LLIN usage by *Plasmodium* infected LLIN owner. Some are unable to use it due to activities they embark upon. However, there was no statistical significance in the activity and *Plasmodium*infection (P>0.01).

It was observed from the study that there was significance (P<0.05) in *Plasmodium*infection based on trimesters. Out of two hundred and ten infected pregnant women, 89(42%), 66(31%), 55(26%) were Plasmodium positive in the first, second and third trimester respectively (Figure 5).

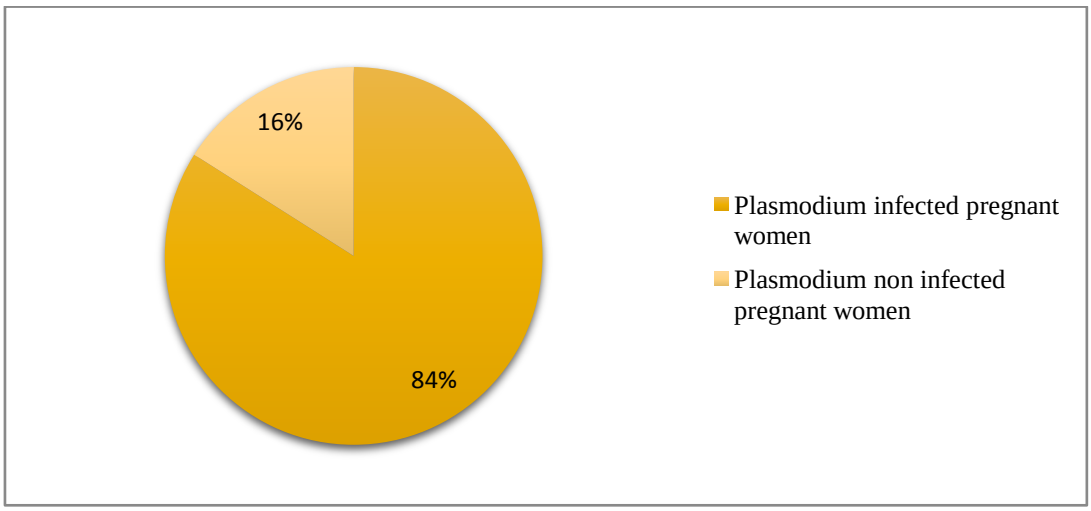

Fig.1. Prevalence of Plasmodiuminfection among pregnant women

Table 1: Prevalence ofPlasmodium Infection among pregnant women and non- pregnant women.

	Pregnant(%)	Non-Pregnant(%)	Total (%)
Plasmodiuminfected	210(70)	22(7)	232(77)
Plasmodium non-infected	40(13)	28(9)	68(23)
Total	250	50	300

Table 2: PlasmodiumInfection rate among Primigravidae and Multigravidae.

PlasmodiumPlasmodiumTotal (%) infected (%)	non-infected(%)		
Primigravidae	121(48.4)	23(9.2)144(57.6)	
Multigravidae	89(35.6)	17(6.8)106(42.4)	
Total	**210**	**40**	**250**

X^2=39.44, df=2

Table 3: Prevalence of Plasmodiumand Anemicity among Pregnant and Non-Pregnant women

		Number anemic(%)	Number non-anemic(%)	Total(%)
***Plasmodium* Infected**	Pregnant	52(24)	158(75)	210
	Non-pregnant	4(18)	18(75)	22
***Plasmodium* non-Infected**	Pregnant	13(33)	27(67)	40
	Non-pregnant	8(29)	20(67)	28
Total		**91**	**80**	**300**

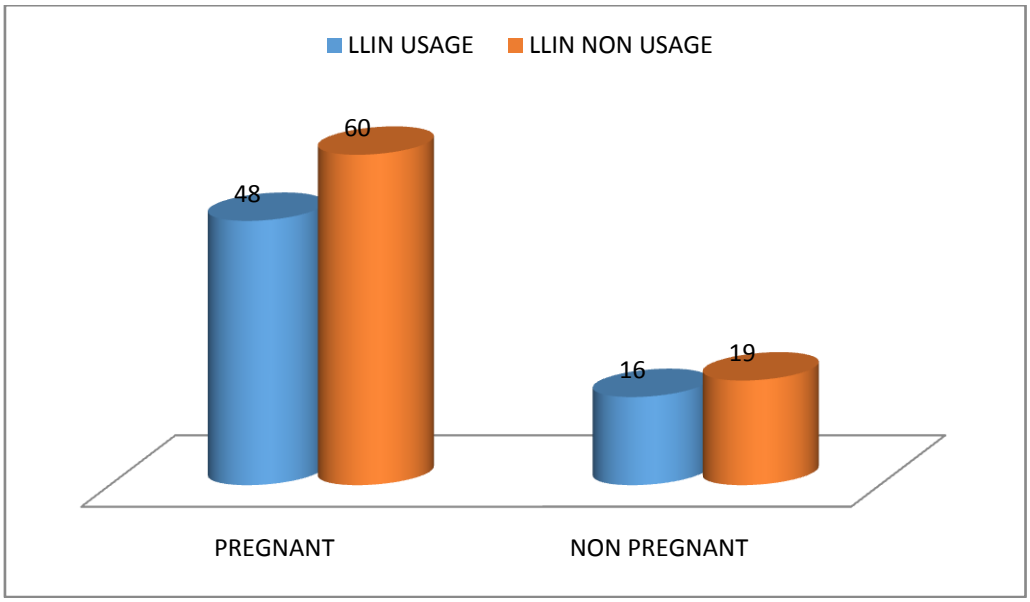

Fig. 2:Attitude of LLIN possessing pregnant and non pregnant women towards the usage
X^2=25.09, df =3, P> 0.005

Table 4: Plasmodium infection in relation to LLIN usage among pregnant and non pregnant women

	Plasmodiumpositive	Plasmodiumnegative	Total
Pregnant women using LLIN	42	6	48
Pregnant women not using LLIN	52	8	60
Non Pregnant using LLIN	6	10	16
Non Pregnant not using LLIN	9	10	19
Total	**119**	**34**	**143**

X^2=31.22df=3

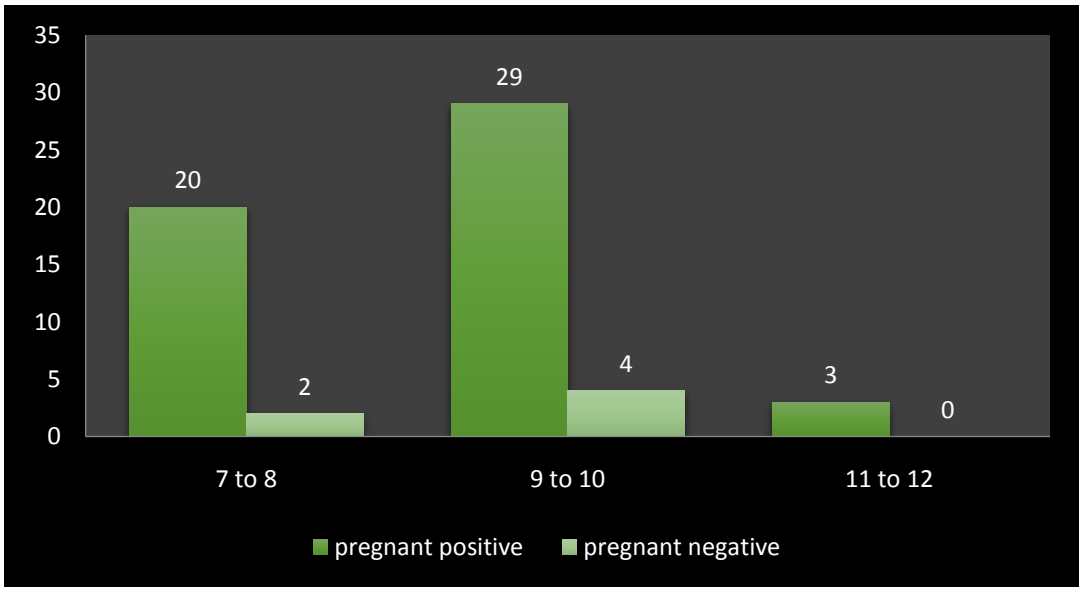

Fig. 3: Plasmodium infection among pregnant LLIN users in relation to sleeping hour.
X^2=2, df=2

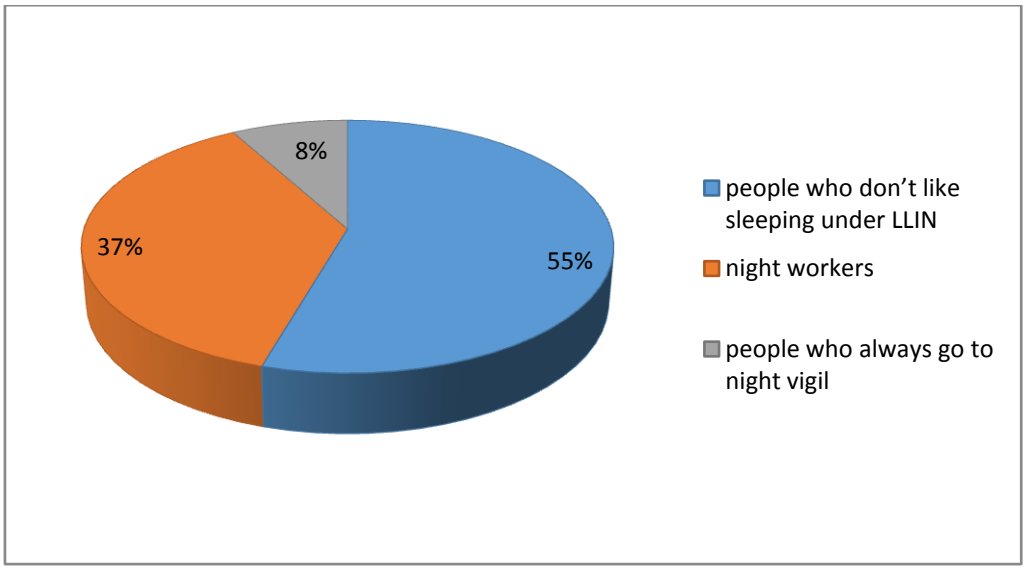

Fig. 4: Factors preventing LLIN usage by plasmodium infected LLIN owners
X^2=9.5 df=2, P>0.01.

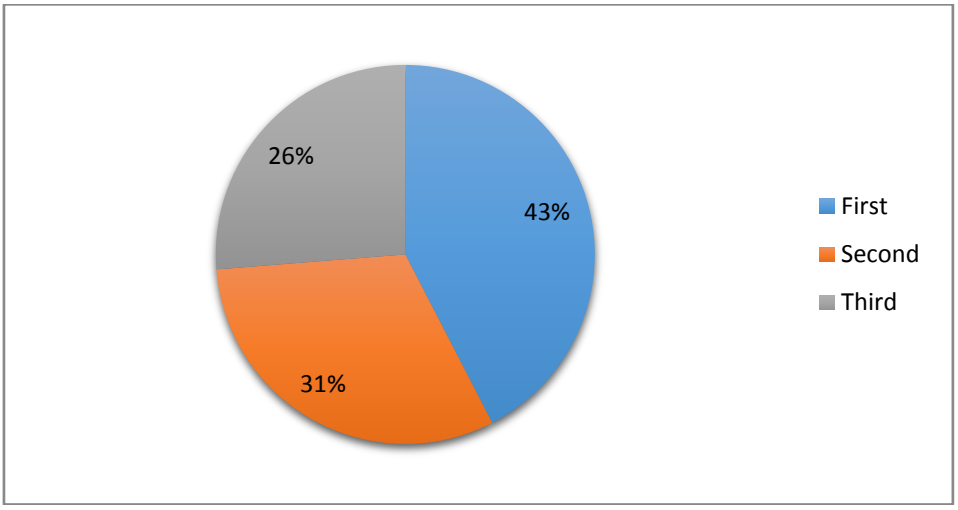

Fig. 5: Prevalence of Plasmodium infection based on trimester
X^2=3, df=4

IV. Discussion

High prevalence of *Plasmodium* infection(84%) was found among the pregnant women attending Tofemedics Diagnostic Centre, Ile-Ife during the time covered by this research (Fig. 1).This rate is in consonance with 80.9% prevalence rate reported by Ohalete *et al.,* (2011). However, it was much higher than between 19.7% and 72% prevalence rate reported by Okwa, (2003); Kagu *et al.*,(2007); Ngele, (2008); Uneke *et al.*,(2008); Agomo *et al.*,(2009).The fact that pregnant women are more attractive to mosquito than non-pregnant individual and that pregnancy is associated with increased susceptibility of pregnant women to malaria as a result of altered immunity may be the reason behind the high prevalence.Lindsay *et al.*,(2000) and Chedraui *et al.*,(2009) also observed high rate of malaria incidence among the pregnant women compared to non-pregnant women.Malaria in pregnancy is dangerous especially when the infection is caused by the most virulent *Plasmodium falciparum* species.The primigravidae (Table 2) found to be more infected in this study is in agreement with other studies (Jeffery *et al.,* 2005; Chukwuocha *et al.,* 2012) and corroborates previous findings that primigravidae are more prone to malaria infection due to sudden reduction in their immune system as a result of sudden change in their physiological and immunological status caused by the pregnancy (McGregor *et al.,* 1983). Likewise, the gestation age was observed to play a role in malaria incidence in this study as women in the 1st trimester were more infected than those in the 2nd and 3rd trimesters. This corroborates previous findings that malaria is most frequent in pregnancy, peaking between 13 and 16 weeks and declining towards term (Guyatt*et al.,* 2004; Chukwuocha *et al.,* 2012).However, itcontradicts the findings of Ohalete*et al.,* (2011) and Idowu*et al.,* (2005) where highest malaria parasites prevalence is found in the second trimester.

Anaemia is one of the pathology of *Plasmodium* infection. The incidence rate of anemia among the *Plasmodium* infected pregnant women is low. This could probably be that the women use haematinics regularly and had enough children spacing which could have allowed them to regain blood and iron loss during the previous birth. The severe anemia observed among one of the control group explains that *Plasmodium* infection is not the only factor which cause anemia in human.

FMoH (2004) recommended the following in the new policy of malaria in pregnancy: Constant use of ITNs/LLINs, IPTp with sulfadoxine pyrimethamine, early detection and prompt appropriate case management of women with symptoms and signs of malaria. Likewise, World Health Organization recommends administration of two or more doses of a safe, effective anti-malarialafter the end of the first trimester to all pregnant women. However, it was discovered from this study that the actual coverage of the use of antimalaria (SP) for IPTp is low.

Although the use of ITNs/LLINs had been reported to reduce malaria incidence, it was observed in this study that high population of the women both pregnant and non-pregnant using the net are still infected with *Plasmodium*. This was majorly caused by the attitude of the women to the use of the net (Fig.4) and the time the women go to bed. Lateness in going to bed would have allowed mosquito bites which present the LLIN as not been effective in reducing malaria incidence. This explains the necessity of the Government to intensify on educating the populace on the advantage of using insecticide bednets. The similarities between the incidence of malaria parasite among the pregnant women who are using insecticide treated nets and pregnant women who are not using insecticide treated net is in contrast to the findings of report of Yahaya and colleagues (2009) who ascribe high malaria parasite prevalence to non-usage of net. This could probably be that the women had been bitten by mosquito before using the net because insecticide bed net actually prevents mosquito bite when used appropriately.

Conclusively, malaria continues to be of economic burden among pregnant women in Ile-Ife, a tropical terrain in Nigeria. Although The Government at both levels had taken bold steps in the effort to reduce malaria incidence among the citizens through free distribution of LLINs, a novel strategy toward reducing mosquito-human contact in the control of malaria is still needed. Malariaologist need to expedite efforts in devising a novel strategy that will incorporate virtually all human activities. Efforts should be intensified in educating pregnant women on ways to protect themselves from infections.

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