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Effectiveness of Insecticide Treated Nets in the Control of Malaria in Tudun Wada, Kano State.Aliyu, I.A.¹ and Yayo, A.M.^{2*}

Department of Medical Laboratory Science, Faculty of Allied Health Sciences, Bayero University, Kano. Kano State, Nigeria¹, Department of Medical Microbiology and Parasitology, Faculty of Basic Clinical Sciences, Bayero University, Kano. Kano State, Nigeria².

Author for Correspondence: murisa682@yahoo.com/+234-

Abstract

Malaria is a major public health problem in Nigeria. Current WHO initiative on malaria control, such as roll back malaria (RBM), emphasizes the use of insecticide- treated net (ITNs), as one of the key strategies for malaria prevention and control. This study was conducted to determine the efficacy of long lasting insecticide- treated nets (LLINs) in the control and prevention of malaria. The assessment was based on differences in malaria incidence, parasite density, and indoor resting densities (IRD) of vector, between the intervention (people using LLINs) and control (not using LLINs) groups. Subjects were given LLINs few months prior to the survey, mosquitoes were collected fortnightly using pyrethroid spray sheet collection method at 4:00-6:00am each day for the period of 6 month. Blood samples were taken for microscopy. The entomological result shows that out of a total of 2386 indoor resting mosquito collected, only 329 were *Anopheles*. Out of which 88 (7.5%) were collected from intervention group, while 241 (19.9%) from control group. The differences in malaria vector density between the two groups were significant ($p \leq 0.05$). A total of 966 slides were examined by Giemsa staining technique. The result of malaria parasite shows 7.2% (17) prevalence of malaria in intervention group, and 15.4% (38) in control group. The result suggested that, the LLINs were effective in malaria control and prevention in the study area. However, the LLINs did not significantly lower the IRD of mosquito. It is recommended that the net impregnation (treatment) protocol be reviewed, and that, people should actively be involved in malaria control, by observing good environmental hygiene. There is also the need for people to be enlightened on personal protection against mosquito bite.

Key words: Malaria parasite, LLINs, Mosquitoes, *Anopheles*.

Introduction

Malaria fever is an infectious disease that is most prevalent in Africa. It is caused by a eukaryotic Protista from the genus *Plasmodium*, which is vectored by female *Anopheles* mosquito. The infection is seen widely distributed to other region of the world. Mortality as a result of malaria are majorly seen in sub-Saharan Africa where over three million death, majority of which in children were reported. Poverty in this region has been attributed to be the main cause of mortality due to malaria. The disease is reported to have kill one African in every 15 seconds, and in Nigeria about 300,000 children died annually due to malaria.

Five species of the *Plasmodium* parasite has been reported to infect humans, the most serious form of the disease is caused by *Plasmodium falciparum*. Other species are *P. malariae*, *P. ovale* and *P. vivax* that are known to cause milder disease in humans. *P. knowlesi* is the fifth species discovered in Asia. It is a zoonotic species known to cause malaria in Macaques and Humans.

Control of malaria depend upon elimination of mosquitoes breeding places, prevention of human-vector contact using screens, pyrethrin-treated netting, protective cloth with sleeves and long trousers, repellents, suppressive drug therapy for exposed persons and adequate treatment of case and carrier. In the 1940s – 1960s spraying the inside surface of houses with a residual insecticide, principally Dichloro-Diphenyl Trichloroethane (DDT), was the main methods by which the incidence of malaria was enormously reduced or almost eradicated, in regions where malaria was endemic. However, due to the declining willingness of government and donor agents to continue funding the spraying programs on a sufficient scale in Africa, there has been reported resurgence of malaria in this region, though not reaching the severity observed in

the 1930s. In the last 3 to 4 decades, treatment of bed nets with residual pyrethroids has become more attractive and applicable than house spray as a means of controlling malaria vector (Lengeler, 1998). For many reasons insecticide-treated nets are expected to be more effective than house spraying in the control of malaria vector, among which is: the fact that most *Anopheles* mosquito are indoor and late-night blood-feeders. Mosquitoes are then intercepted as they approach sleepers in search of blood. Thus, nets may be considered insecticidal traps baited with the order of the sleeper inside (Curtis and Mnzava, 2000). However, *Anopheles* mosquitoes may not rest long enough on the insecticide sprayed wall and ceiling to have picked up the lethal dose, while in DDT and other irritant insecticide may even result in the mosquitoes shortening their resting time, making the matter worse. And also, the possibility of replastering the walls after being sprayed (Curtis and Mnzava, 1998) thus covering up the insecticide deposit.

Furthermore, Insecticide Treated Nets (ITNs) were reported to be more effective in the prevention of malaria than traditional bed nets (Jones, 2000) since the latter only provide partial protection as the net allows mosquitoes to enter and feed, especially, if the net is even slightly torn or not tucked in properly (Jones, 2000). However, if they were treated with insecticides, the nets can be very effective at repelling and killing *Anopheles* mosquito (Salako, 2002; Lines *et al.*, 1987). It has been reported that ITNs with holes are still effective in repelling mosquitoes. Carnervale *et al.* (1992), indicated that the insecticide can affect the mosquito as they work their way through. So far, the use of ITN has led to reduction in child mortality ranging from 14 – 72% in some African trials (Webster, 2000). On the other hand, for insecticide-treated nets to be fully effective, active involvement of community members may be required to make sure that nets are used, even during season when their use is uncomfortably hot and there may not be enough biting by nuisance insects to make net use seem worthwhile, though there may still be enough dangerous vectors about (Curtis and Mnzava, 2000).

In Nigeria there are nearly 110 million clinically diagnosed cases of malaria annually, accounting for 60% of outpatients' visits and about 30% of hospitalization, resulting in about N132 billion (about \$900 million) lost to malaria annually in prevention, treatment cost and productivity loss (UNICEF, 2010). Nigeria has renewed its commitment through the National Malaria Control Program and the Roll-Back Malaria partners to

reduce the malaria burden by half, by the end of the year 2010. One of its strategies is to distribute 63 million long lasting insecticide-treated mosquito nets to 32 million households in all 36 States and the Federal Capital Territory. The campaign is the largest distribution of LLINs in the world aimed at reducing vector – human contact (UNICEF, 2010). This study was designed to assess the impact of these LLINs on control of malaria in Tudun Wada LGA, Kano State, Nigeria.

Materials and Methods

The study area is Tudun Wada Local Government Area, one of the 44 local Governments of Kano State. It is about 100km South way from Kano, the most populous and commercial city of Nigeria. The town lies at latitude 11°58' and longitude 08°26' with mean annual rainfall 787-989mm. Rainy season usually covers the period between June to September. The study area comes under open woodland Sudan savannah (Figure 1). The study was conducted at Tudun Wada main town (Sabon Gari and Tsohon Gari) and Rugu Rugu. The population of the area is relatively homogeneous, made mostly of Hausa and Fulani. The primary economic activities in the area is subsistent farming, and in the case of Rugu Rugu with fishing activities. All members of the selected households in both intervention and control group who consented to participate in study were included in the survey.

Ethical clearance was obtained from the Local Government Medical Department, after ethical defence, and following due ethical process before commencement of the research. Consent form was given to each member or guardian of participating randomly selected household, to seek their written informed consent after which the questionnaire was then administered for their responses.

Randomized control trial study design was employed, in which the study was designed in two groups, experimental /intervention group (using LLINs) and control group (not using LLINs). Thus, the two groups were as alike as possible in all respect, except in the use of net (LLINs). All subjects were at the same level of exposure and risk factors. The only observed differences between the two groups were attributed to the real effect of LLINs. The effectiveness of LLINs in malaria control were measured as the proportion of malaria cases, level of parasitaemia (parasite densities) and mean mosquito densities in the control group, that would not have occurred had they slept under nets (LLINs).

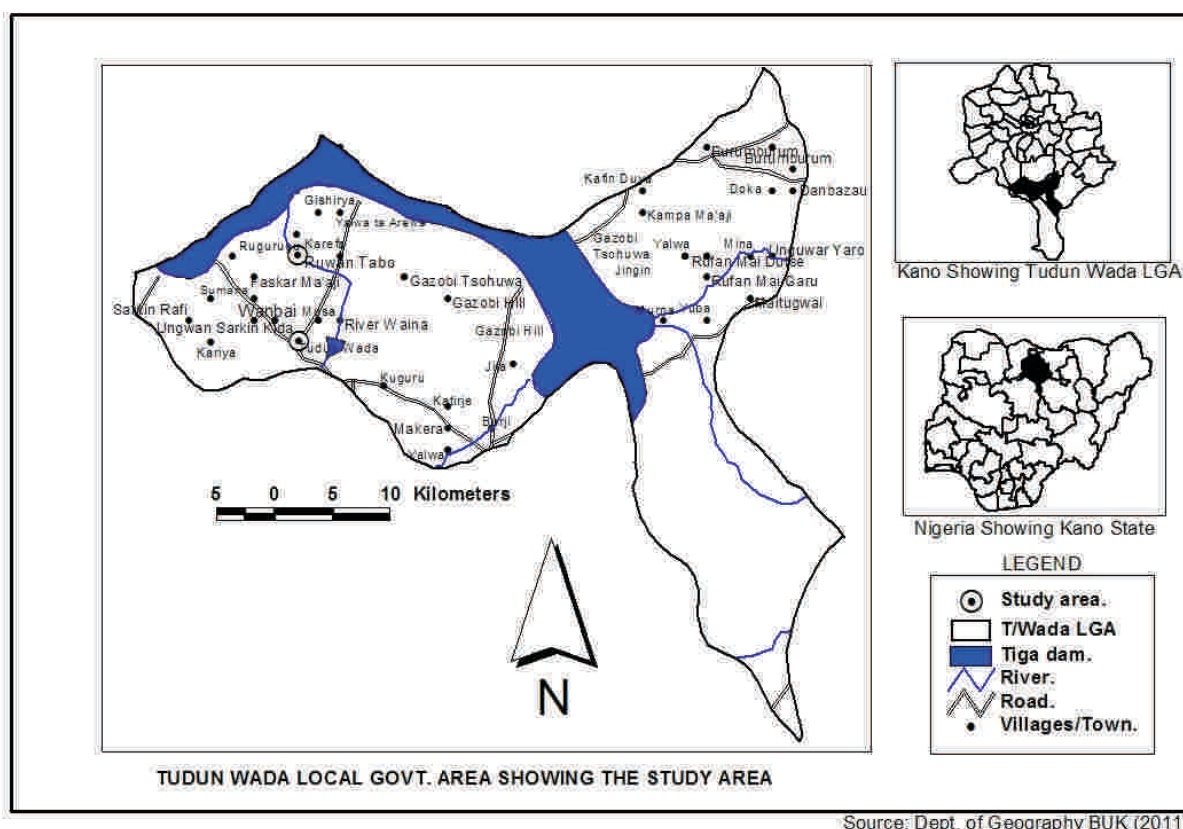


Figure 1: The map of Tudun Wada showing the study area

Mosquito collection

Indoors mosquitoes were collected using insecticide spray sheet collection methods (Oyewole *et al.*, 2007; WHO, 1975) from 30 randomly selected houses (dwellings) assigned to either intervention or control group. The collection was done forth-nightly for the period of six month (July-December 2010), which was considered fair enough to observe the impact of the LLINs in dry and rainy seasons. The collection was done between 4:00am to 6:00am each day of collection. The collected mosquitoes were sorted and identified using morphological features with aids of identifications manual using hand magnification lens (Gillies and Coetzee 1987; WHO, 1975). Mosquito species were identified; blood fed mosquitoes were sorted, counted and recorded each day of the collection. Their gender was also determined according to Gillies and De Meillon, (1968). Mean mosquito density were calculated as a total number of mosquito caught in the household by the total number of individual sleeping in each room.

Blood collection and staining

Capillary blood was collected aseptically, thin and thick film was made according to Cheesbrough, (2000). The slides were stains by Giemsa staining

techniques and were examine accordingly. Malaria parasite density was calculated according to Greenwood and Armstrong, (1991). Average numbers of malaria parasites, in about 10 to 50 fields were taken, depending on parasite load, from thick films, which was multiply by factor of 500 to get the parasite densities.

Result

A total of 2386 indoor resting mosquitoes were collected from control and intervention groups throughout the study period. Out of which 1192(49.1%) were collected from intervention group and 1214(50.9%) (Table 1) were collected from control group. There was no statistical significant difference in the total number of mosquitoes collected from intervention group compared with control group by student t-test (Table 2). However, 53 (5.5%) of the total mosquito collected from intervention group were identified as *Anopheles* compared with 105 (9.6%) anopheles identified in the control group (Table 2). The result of malaria vector density in control group were significantly higher than that of intervention group when compared statistically by student t-test at 95% confidence level ($p < 0.05$). Similarly, the number of blood fed

Anopheles mosquitoes collected from the control group 37(3.0%) were higher than that collected from intervention group 15(1.3%) (Table 2). While 216(18%) blood fed mosquitoes from intervention and 312(25.7%) from control group were either *aedes* or *culex* (non-anopheles) (Table 2). Two species of

Anopheles were identified in the study area these are *Anopheles gambiae* and *Anopheles funestus*. Nonetheless, *Anopheles gambiae* was the most predominant species identified in the study area during the study time, which is 80.2% of the total *Anopheles* collected (Table 4).

Table 1: Comparison of monthly mosquito collection, species variation of malaria vector for both control and intervention communities.

Months	Total number of mosquitoes collected	Non- <i>Anopheles</i>	Number of <i>Anopheles</i> mosquitoes collected		Number of blood feed <i>Anopheles</i> mosquitoes
			<i>An. gambiae</i>	<i>An. funestus</i>	
July	440	416	24	21	17
August	580	538	42	36	20
September	603	589	24	22	11
October	555	554	12	11	1
November	253	252	5	5	1
December	132	131	1	0	1

Table 2: Difference in mosquito density between intervention and control group.

Group	Total No. of Mosquito collected		Total No. of Blood fed Mosquito collected	
	<i>Non-Anopheles</i>	<i>Anopheles</i>	<i>Non-Anopheles</i>	<i>Anopheles</i>
Intervention group	1172 (49.1%)	38 (3.2%)	216 (18.4%)	15 (1.3%)
Control group	1214 (50.9%)	68 (5.6%)	312 (25.7%)	37 (3.0%)
Total	2386	106	528	52
<i>P value</i>	0.303(NS)	0.04*	0.05	0.00**

Key

** indicates highly significant.

* indicates significant. No. = number

NS= indicates not significant.

Intervention group = people using LLINs.

Control group = people not using LLINs.

Table 3: Seasonal differences of mosquito density between intervention and control communities.

Season	Intervention group	Control group	Total
Rainy season	689	794	1483
Dry season	483	420	903
Total	1172	1214	2386

Key

Intervention group = people using LLINs

Control group = people not using LLINs.

Table 4: Mosquito species identified from intervention and control group

Subjects	<i>Anopheles gambiae</i>	<i>Anopheles funestus</i>	Non-anopheles
Intervention group	31	7	1172
Control group	62	6	1214
Total	93	13	2386

Key

Intervention group = people using LLINs.

Control group = people not using LLINs

Parasitological findings

A total of 966 blood specimens were collected for parasitological studies, 483 (50%) of the sample were collected during raining season, and 483 (50%) were collected during the follow up survey in dry season to compare the prevalence and density of malaria during dry and rainy season. Approximately half of the samples (237) were collected from intervention group and the other half (246) were collected from control group during each survey (Table 5).

The result of this study showed an overall malaria prevalence of 7.2% in the intervention group, however in control group the overall prevalence was 15.4%. The result was compared statistically by chi-square and the difference between the two was statistically significant ($p < 0.05$) (Table 5).

Trophozoite stage of *Plasmodium falciparum* were the dominant malaria parasite form observed (Figure 2) under the microscope (94.5% of the total positive slides), gametocytes and malaria pigment were 1.8%, and 3.6% respectively of the total positive slides examined, and no schizontes stage were seen (Table 6). Furthermore, *Plasmodium falciparum* was the only species of malaria parasite seen during the study period in the study area (Table 7). Comparison of the mean malaria parasite densities between intervention and control groups showed statistically significant difference ($p < 0.01$), with overall malaria parasite densities higher in control group than the intervention group.

The prevalence of malaria parasite during rainy season was seen to be higher than that observed during dry season ($p < 0.01$) (Figure 3).

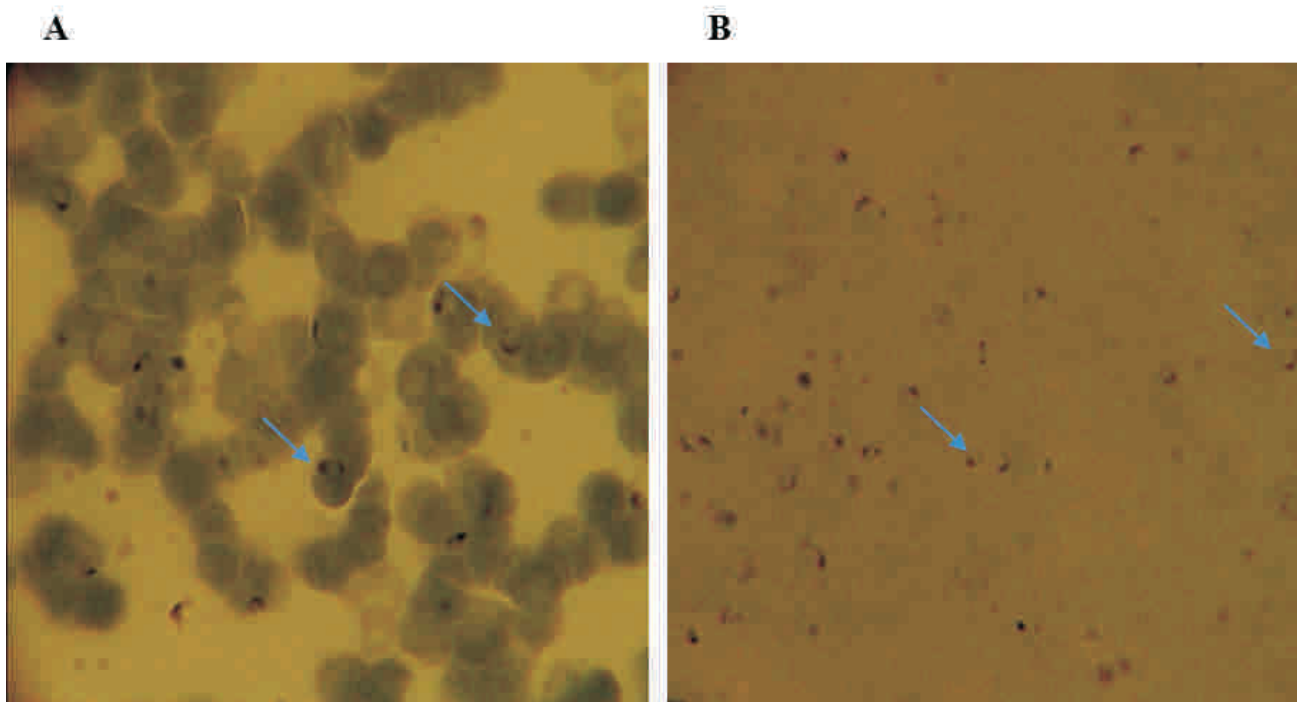


Figure 2: Microscopic view of Plasmodium falciparum trophozoite (A) Giemsa stained smear of a thin blood film, the arrow showed trophozoite of malaria parasite inside red blood cells. (B) Giemsa stained smear of a thick blood film, the arrow showed trophozoite form of Plasmodium falciparum outside (in lysed) blood cells.

Table 5: Incidence of malaria in control and intervention groups

Subject	Intervention group		Microscopy results		Total
			Malaria parasite seen	No malaria parasite seen	
		Malaria parasite count	17	220	237
		% within subject	7.2	92.8	100
	Control group	Malaria parasite count	38	208	246
		% within subject	15.4	84.6	100
		Total count	55	428	483
	Total	% within subject	11.4	88.6	100

Key:

Intervention group = people using LLINs

Control group = people not using LLINs

Table 6: Different stages of malaria parasite observed by microscopy in intervention and control group

Malaria parasite			Subjects		Total
			Intervention group	Control group	
Trophozoite	Count		17	35	52
		% within microscopy result	32.7	67.3	100
	Gamatocytes	Count	0	0	0
		% within microscopy result	0.0	0.0	0.0
	Pigment	Count	0	0	0
		% within microscopy result	0.0	0.0	0.0
Total	Count	17	38	55	
	% within microscopy result	30.9	69.1	100.0	

Key:

Intervention group = people using LLINs.

Control group = people not using LLINs.

Table 7: Impact of insecticide treated nets(ITNs) on malaria prevalence and malaria parasite density

Subjects	Malaria parasite seen	No malaria parasite seen	Malaria parasite stage	Malaria parasite species	Average parasite density
Intervention group	17(7.2%)	220(92.8%)	TR. (100%)	<i>P falciparum</i> (100%)	6,500/ml
Control group	38 (15.4%)	208 (84.6%)	G.0%, P.0% TR. (100%)	<i>P falciparum</i> (100%)	19,500/ml
Total	55	428	G0.0%, P.0.0%		
p- value	≤ 0.05*				≤ 0.00**
% Protection	38.2%				

Key:

TR= Trophozoite

G. = Gametocytes, P.= Malaria pigment,

** indicates high significant difference,

* indicates significant difference,

No. = Number

Intervention group = people using LLINs.

Control group = people not using LLINs.

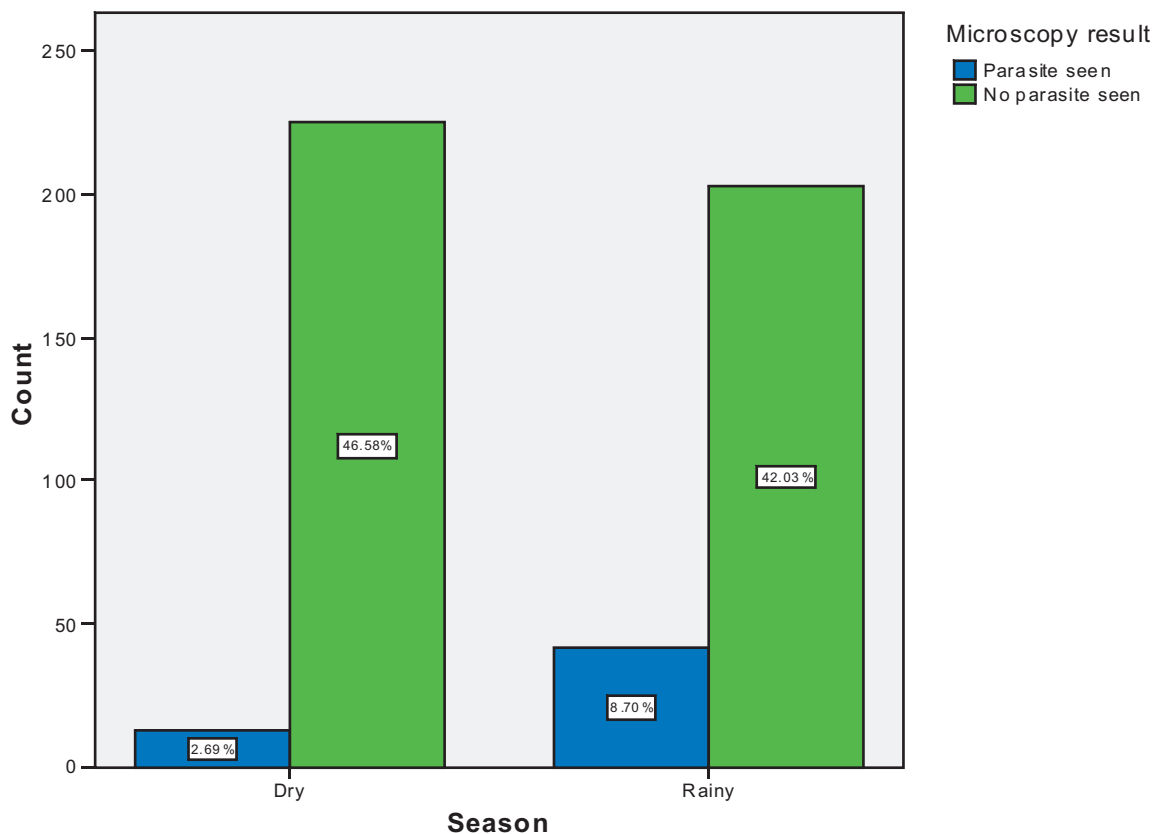


Figure 3: Prevalence of malaria during dry and rainy season

Discussion

Entomological findings

Entomological findings show that the LLINs distributed in the study area does not have much impact in reducing total mosquito densities, when the total number of mosquitoes collected from intervention group were compared statistically with that of control group by student t-test the result shows no significant difference. The implication of these finding may mean that the LLINs is not as effective as it is thought. The result of the present study is in concordance with previous reports (Himeidan *et al.*, 2007; Prapanthadara *et al.*, 2002) which indicated that some mosquito species were found resistant to Permethrin, one of the chemicals used in treating LLINs. However, the result of the present study is at variance with a previous report in Tanzania (Tami *et al.*, 2004) and in Sudan (Hassan *et al.*, 2008) which indicated that the net treated with Permethrin were very efficacious and can achieve up to 52% to 63% reduction in malaria burden respectively.

The mean malaria vector (*Anopheles* only) density in the control group were 105(9.6%), this is higher than that of intervention group 53(5.5%). This result indicates the effect of the net in reducing malaria vector density in the intervention group. However, there was no significant difference in the total mosquitoes caught (*Anopheles* and non-*Anopheles*) between the two groups. Which may indicate that the net is not very effective in killing the mosquito, but only serves as host-vector barrier, a role played by traditional bed net. Nonetheless, the blood fed total caught mosquitoes (both non-*Anopheles* and *Anopheline*) was more in the control group than in intervention group. These differences might be as a result of LLINs usage in the intervention groups, which shouldn't have been there, had the control group use the net.

All *Anopheline* mosquitoes collected fall into 2 major groups of *Anopheles* mosquito: *Anopheles gambiae* (87.7%) and *Anopheles funestus* (12.3%). This result is in concordance with reports from other parts of Nigeria (Osundu and Jerume, 2009; Oyewole *et al.*, 2007) which showed that *Anopheles gambiae* and *Anopheles funestus* constituted the largest proportion of malaria vector in Nigeria. The reports described Nigeria as a malaria endemic state due to higher level of human-vector contact, consequent to higher vector density especially in the southern part of the country where population of *Anopheles* were significantly higher because of the heavy rain fall compare to the northern part, hence, there were dense vegetation and many small bodies of water around or within human habitation, couple with poor hygienic habit of leaving grasses around human dwellings.

In the present study, higher seasonal variation in malaria vector density was recorded; *Anopheles* mosquitoes were collected between July and September with peak collection in August. This coincided with rainy season in the study area and tends to disappear almost completely during dry season which (coincided with cold and dry weather in the area).

Parasitological findings

In the present study we observed a 38.2% reduction in malaria prevalence in the intervention group compared with the control group. This result is in concordance with Osundu and Jerume (2009) who found a reduction in incidence of malaria in intervention communities. They reported that the insecticide- treated nets provided 68% (34/50) protection compared with communities not using LLINs. Similarly, Hawley *et al.* (2003) reported 70% malaria protection in communities using insecticide-treated nets in Western Kenya. Eisele *et al.* (2010) reported 48.1% reduction in malaria related child mortality in community using insecticide- treated nets compared with control group. However, the result of the present study showed lower protection level than what was reported in other LLIN efficacies studies, which may indicate the need to re-evaluate nets treatment strategies and of course the chemical used in the treatment.

The prevalence of malaria was much higher during the rainy season compared with dry season ($p < 0.01$) (figure 3). This is alarming since current climatic changes (global warming/ greenhouse effects), result in an increased volume of water and extended period for raining season, which may result in an increased malaria vector breeding ground and vector density that may lead to increased vector density and hence increased prevalence of malaria, maternal and child mortality.

Conclusion

The net has assisted in reduction of malaria parasite incidence in the study area by at least 38.1%. However indoor resting density of malaria vector was not significantly reduced in the intervention group compared with control group, indicating mosquito resistance to the chemical used in the treatment of the LLINs in question. To have an effective malaria control in the study area, people with the nets should actively be involved in malaria control, by making sure that the nets are used appropriately. When the need arises, they should use insecticide even if they are using nets. Stakeholders should review the impregnation procedure and chemical type and concentration use in net treatment. People should be enlightened on the preventive and control measures against malaria. People infested should be identified and treated promptly to avoid spread of malaria.

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