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ORIGINAL ARTICLE

LONG-LASTING INSECTICIDE-TREATED NETS (LLINS) USED TO REDUCE THE INCIDENCE OF MALARIA IN A MUNICIPAL DISTRICT OF THE BRAZILIAN AMAZON

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ABSTRACT

Malaria is still the parasitic disease with the greatest worldwide impact. Recently in Brazil, almost all cases of the disease have been recorded in the Amazon region. The struggle against the vectors through insecticide treated nets associated with rapid diagnosis and treatment, is currently the main strategy. This study aimed to determine the protective effect of these devices on the incidence of malaria in one municipality in the Amazon, and was conducted with information from the Brazilian Ministry of Health and with the analysis of 10,050 slides of thick blood smears that were prepared and examined *in loco*. The Shapiro-Wilk test was used to determine the normality of the data and the Mann-Whitney test was used for all comparisons of the analyzed variables. The significance level was set at $p \leq 0.01$. The results revealed a significant reduction in the number of malaria cases in all analyzed variables, including the species of the parasite, the level and type of infection, and the gender and age of the diagnosed individual ($p<0.01$). These findings confirmed that these devices are an important tool for disease control, presenting a new variable in combating the disease in the studied population. Therefore, the use of insecticide-treated nets is recommended as a preventive measure and guidance to the population regarding the correct way to use this device is required to avoid problems such as the loss of naturally acquired immunity, reduction in the protective effect of the device and the development of resistance to the insecticide.

KEY WORDS: Malaria; Amazon; vectors; insecticide-treated bednets; LLINs.

RESUMO

O uso de LLINs (long-lasting insecticide-treated nets) ajuda a reduzir a incidência de malária em um município da Amazônia brasileira

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A malária continua sendo a doença parasitária mais impactante em todo o planeta. No Brasil, quase todos os casos são registrados na região amazônica. O combate ao mosquito vetor da doença por meio da utilização de mosquiteiros impregnados com inseticidas de longa duração, associado ao diagnóstico rápido e ao consequente tratamento dos doentes, é apontado, atualmente, como importante estratégia de enfrentamento da malária. Este estudo foi realizado entre os anos de 2012 e 2013 com informações obtidas do banco de dados do Ministério da Saúde do Brasil e da leitura de 10.050 lâminas de gota espessa sanguínea, preparadas e examinadas *in loco*, conforme o método de Walker. Para a determinação da normalidade dos dados foi usado o teste de Shapiro-Wilk. Para amostras independentes, utilizou-se o teste de Mann Whitney em todas as comparações das variáveis avaliadas. Foi admitido um nível de significância de $p \leq 0,01$. Os resultados apontaram uma diminuição significativa dos casos de malária em todas as variáveis avaliadas, como a espécie de parasito envolvida, o nível e o tipo de infecção ou o sexo e a idade do indivíduo diagnosticado ($p< 0,01$). Desse modo, ficou confirmado que esses mosquiteiros impregnados constituem importante instrumento de controle da doença, uma vez que representaram a variável nova inserida no contexto do combate à doença na população pesquisada. Recomenda-se, portanto, a utilização desses equipamentos como medida preventiva no combate à malária. Contudo, ressalta-se a necessidade de orientação sobre a forma correta de seu uso pela população para que sejam evitados problemas como a perda da imunidade naturalmente adquirida, a diminuição da efetividade protetiva do equipamento e o aparecimento de resistência ao inseticida.

DESCRITORES: Malária; Amazônia; insetos vetores; mosquiteiros tratados com inseticidas.

INTRODUCTION

Malaria is the parasitic disease with the greatest worldwide impact. According to World Health Organization (WHO) estimates, approximately 198 million new cases were recorded in 2013, resulting in 584 thousand deaths (WHO, 2014).

The disease resulting from this infection has a classical clinical presentation consisting of episodes of chills followed by high fever, malaise, nausea, headaches, and pain in the joints. Malaria is caused by several species of protozoa of the genus *Plasmodium*, with *P. falciparum* being the most prevalent species (Nadjm & Behrens, 2012), which is usually transmitted by an infected female mosquito of the genus *Anopheles*.

Notably, at the end of the 1980s, access to malaria prevention and control measures increased with the installation of long-lasting insecticide-treated nets (LLINs) impregnated with insecticides such as deltamethrin, which belongs to the pyrethroid group of insecticides (Santos et al., 2007). The results were satisfactory (Carnevale et al., 1992) and the use of these nets spread through distribution in endemic areas around the world. The National Program for Malaria Control in Brazil currently applies key combat strategies as well as the rapid and accurate diagnosis of infections and treatment of patients with the disease, and as a complementary measure the distribution of LLINs (Brasil, 2013).

At the end of 2011, the Brazilian Ministry of Health provided financial support for the purchase and distribution of LLINs to certain municipalities located in the Legal Amazon, which is an area that records almost all of the registered malaria cases in the country. Several municipalities adjacent to the Trans-Amazonian highway received this assistance, including the municipality of Pacajá in the state of Pará (PA), which received 16,850 LLINs.

Following WHO guidance, mosquito nets impregnated with deltamethrin were first distributed in January 2012 as a preventive measure against the disease. However, two years after these mosquito nets were handed out, neither scientific evidence nor an epidemiological analysis was available to determine the efficacy of this device in preventing and controlling malaria in the municipality of Pacajá-PA.

Considering the lack of evidence on the efficacy of this device in Pacajá-PA, this study aimed to determine the protective effect of LLINs as an auxiliary method with respect to the incidence of malaria in the municipality of Pacajá and the epidemiological profile of this infection before and after the installation of these nets.

MATERIALS AND METHODS

This study was conducted in 2012 and 2013 in the municipality of Pacajá, which is located in the southwestern mesoregion of the state of Pará, Brazil, adjacent to the Trans-Amazonian Highway (BR 230). This municipality has a population of about 40 thousand inhabitants.

The investigation was a descriptive cross-sectional study with the first phase consisting of an epidemiological survey using data obtained from the database in the Epidemiological Surveillance System for Malaria (*Sistema de Informação da Vigilância Epidemiológica-Malária* SIVEP-MALARIA) in the Brazilian Ministry of Health between 2010 and 2011 (before the installation of the LLINs). The second phase of the study was conducted between 2012 and 2013 (after the distribution and installation of the LLINs) and consisted of 10,050 slides for malaria diagnosis and *Plasmodium* species identification, which were prepared and examined *in loco*, according to the method described by Walker (Brazil, 2005). The mean malaria prevalence was calculated from the data obtained in these two phases, and epidemiological characteristics of the disease in the population studied were compared.

The thick smear technique was applied to diagnose malaria and to identify the *Plasmodium* species. For preparing the thick smears, drops of peripheral blood were collected from the fingertips of the participants, placed on glass slides, and smeared (square-shaped, two smears per slide). After drying, the slides were immersed in methylene blue for two seconds for dehemoglobinization, and then stained with 2% Giemsa/phosphate buffer

solution for 10 minutes. Subsequently, the slides were washed with phosphate buffer, dried, and viewed under an optical microscope, with an immersion objective, according to the method described by Walker.

This study was submitted to and approved by the Human Research Ethics Committee of the Institute of Health Sciences, Federal University of Pará (Universidade Federal do Pará), under number 351,836, and it is in agreement with the guidelines and standards recommended by Resolution Nº. 466 (from December 12, 2012) of the National Health Council.

The BioEstat 5 software was used for the statistical analysis. The Shapiro-Wilk test was used to determine the normality of the data. The Mann-Whitney test was used for independent samples in all comparisons of the variables analyzed between 2010-2011 and 2012-1013 as the data were not normally distributed. Thus, the data are expressed as medians and interquartile ranges. The percent difference was calculated using the following equation: $\Delta\% = \{\text{Group 1} - \text{Group 2}\}$. Statistical significance was set at $p \leq 0.01$ with an error (α) of 1%.

RESULTS

16,500 LLINs were installed between January and March 2012, and the incidence of malaria cases significantly decreased ($p<0.0001$) two years after their installation in comparison with two years before their installation (Table 1), even considering the reduced number of tests performed over the study period ($p<0.01$).

Table 1 also shows that the reduced incidence of malaria infections was significant at all levels of parasitemia ($p<0.01$). The reduction in positive slides was $\Delta\% = -215.5\%$. The reductions for the cases < ½ and < ½ + were $\Delta\% = -50\%$ and $\Delta\% = -17\%$, respectively. In addition, as presented in Table 1, the cases (+; $\Delta\% = -18\%$); (++; $\Delta\% = -127.5\%$); (+++; $\Delta\% = -13\%$); and (++++; $\Delta\% = -7\%$) were also statistically reduced ($p<0.01$) between the studied periods.

There were no cases of disease with a parasitemia level of four crosses (++++) recorded after the use of LLINs. Notably, this is the highest and more dangerous level of parasitemia, especially in the disease type known as malignant tertian, which is caused by *P. falciparum*.

Figure shows the results of positive malaria cases by gender. The analysis also revealed a significant reduction of cases in the period between 2012 and 2013 for both males ($\Delta\% = -147.5\%$; $p<0.0001$) and females ($\Delta\% = -70.5\%$; $p<0.0001$), compared with the period between 2010 and 2011.

Table 1. Comparison of the mean number of malaria cases diagnosed between 2010 and 2011 (before the implementation of malaria prevention measures using LLINs) with cases diagnosed between 2012 and 2013 (immediately after the use of these nets), using plus signs to classify the level of parasitemia.

Variables	Examined Slides	Positive slides	<1/2	1/2+	+	++	+++	++++
Period	Md (IR)	Md (IR)	Md (IR)	Md (IR)	Md (IR)	Md (IR)	Md (IR)	Md (IR)
2010-2011	1.010 (325.5)	275.5 (101.7)	63 (38)	21 (15.5)	25 (15.5)	158.5 (68.7)	15 (8.5)	7 (0.1)
2012-2013	457 (222)	60 (48)	13 (8.7)	4 (2)	7 (4.5)	31 (26)	2 (1.2)	0 (0)
p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.01

Md= Median; IR= Interquartile Range.

Source: Period 01/2010 to 12/2011: SIVEP/Malaria/Brazilian Ministry of Health; Period 04/2012 to 12/2013: Slides examined after an active search. Blood slide examination: < 1/2 (less than 40 parasites counted in 100 examined microscopic fields); 1/2+ (40 to 60 parasites counted in 100 examined microscopic fields); + (1 parasite counted in 1 examined microscopic field); ++ (2 to 20 parasites counted in 1 examined microscopic field); +++ (21 to 200 parasites counted in 1 examined microscopic field); ++++ (more than 200 parasites counted in 1 examined microscopic field).

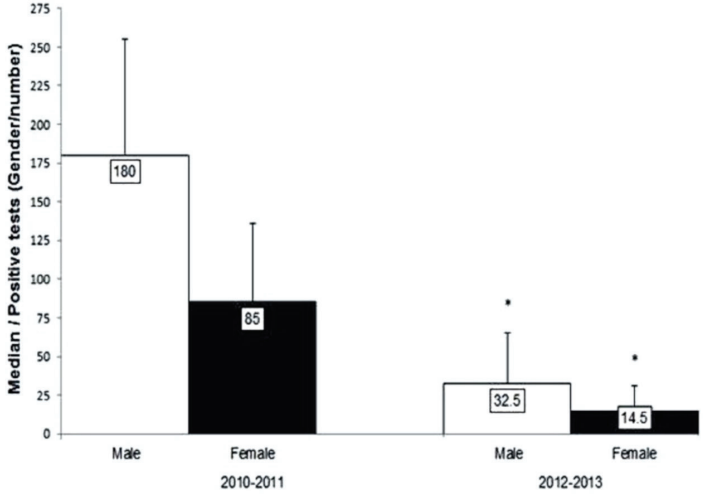

Figure. Results of positive malaria cases by gender. The analysis also revealed a significant reduction of cases in the period between 2012 and 2013 for both males (Δ% = -147.5%; p < 0.0001) and females (Δ% = -70.5%; p<0.0001), compared with the period between 2010 and 2011. The symbol (*) indicates p<0.01.

As shown in Table 2, the distribution of malaria per *Plasmodium* species responsible for infection and diagnosed in the periods studied also exhibited a significant reduction ($p<0.0001$).

Table 2. Distribution of malaria cases before and after the implementation of malaria prevention measures using LLINs, according to the *Plasmodium* species

Variables	F	V	F+V	M	O
Period	Md (IR)	Md (IR)	Md (IR)	Md (IR)	Md (IR)
2010-2011	33.5 (16.5)	244 (102.7)	2 (1.5)	0	0
2012-2013	6 (3.1)	47 (37)	0 (0.1)	0	0
p-value	< 0.0001	< 0.0001	0.002	-	-

Md = Median; IR = Interquartile Range.
Source: Period 01/2010 to 12/2011: SIVEP/Malaria/Brazilian Ministry of Health; Period: 04/2012 to 12/2013: the slides were examined after an active search.
F (*Plasmodium falciparum*); V (*Plasmodium vivax*); F+V (mixed *P. falciparum* and *P. vivax* infection); M (*Plasmodium malariae*) and O (*Plasmodium ovale*).

There was also a significant reduction ($p<0.0001$) in infections caused by *P. falciparum* (Table 3) in all age groups.

Table 3. Cases of *Plasmodium falciparum* malaria before and after the implementation of malaria prevention measures using LLINs, according to patient age groups.

Age Group	Child	Adult	Elderly	Total
Period	Md (IR)	Md (IR)	Md (IR)	Md (IR)
2010-2011	76.8 (27.8)	127.5 (70.3)	20.5 (12.4)	224.8 (90.3)
2012-2013	15 (10.5)	36 (15)	5 (3.2)	56 (22.1)
p-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Md = Median; IR = Interquartile Range.
Source: Period 01/2010 to 12/2011: SIVEP/Malaria/Brazilian Ministry of Health; Period: 04/2012 to 12/2013: the slides were examined after an active search.
Child (aged 6 months to 14 years); Adult (aged 15 to 49 years); Elderly (50 years and older).

DISCUSSION

Malaria has been shown to be the main health problem in several populations worldwide. In Brazil, almost all cases of the disease (99.7%) have been recorded in the Amazon region (Brasil, 2013). Several measures have been taken, following WHO recommendations, to control the chain of malaria transmission, including indoor residual spraying; treatment with different combinations of drugs; larval control (Carnevale et al., 1992; Ursing et al., 2014; WHO, 2014) and, currently, the use of LLINs, which have yielded excellent results in several countries (Joshi et al., 2003; Karch et al., 1993; Ursing et al., 2014; Zhang & Yang, 1995). According to the United Nations Children's Fund (UNICEF) report, the use of LLINs saved the lives of 125 thousand people in ten African countries between 2001 and 2007 (UNICEF, 2015).

LLINs impregnated with deltamethrin have been used in the Amazon, where malaria is endemic, but the results have been contradictory. Some studies indicate low protective effect of LLINs (Santos et al., 1998), while others show a high protective effect of these devices (Xavier & Lima, 1986).

In the present study, the median values of malaria infections were compared before and after the installation of LLINs for malaria prevention in the municipality of Pacajá-PA, Brazilian Amazon region. The results showed a significant decrease in all levels of parasitemia in the disease ($p<0.01$), including malaria with two crosses (++), which, according to the Brazilian Ministry of Health classification, is the most common level of malaria infection parasitemia observed in Amazonian cities (Brazil, 2013). The parasitemia level with four crosses (++++), which is particularly severe for the infection caused by *P. falciparum* (Noronha et al., 2000) was already low before the installation of LLINs and was no longer observed after their use. This decrease in total cases of the disease, should also be associated with other variables, such as rapid diagnosis by experienced microscopists and the immediate and correct treatment of patients, which are fundamental measures described in the National Plan to Combat Malaria in Brazil, and performed in the studied municipality (Oliveira-Ferreira et al., 2010)).

Malaria is a wide spectrum disease, ranging from subclinical or asymptomatic cases to toxemic cases, which can progress to a severe condition and even to death if not specifically and adequately treated in a timely manner also depending on the level of parasitemia and the *Plasmodium* species.

Due to its shorter life-cycle in different tissues, the elevated production of merozoites during both tissue and erythrocytic schizogony, and its ability to infect red blood cells of any age, *P. falciparum* can promote hyperparasitemia, which is closely related to the severity of the infection. Moreover, *P. falciparum* is the only species that clearly alters microcirculation, contributing to worsening the disease. Therefore, most cases of severe malaria and death in

several places worldwide are caused by *P. falciparum*, although there is also a report of severe infection caused by *P. vivax* (Gomes et al., 2011). Therefore, these severe forms of the disease require rapid and appropriate actions for patient care.

The reduction in the number of cases of malaria infection with high levels of parasitemia, and the absence of records of these high levels, in cases of *P. falciparum* malaria after the use of LLINs in all age groups ($p<0.0001$), may indicate the protective effect of this device against the species that cause the vast majority of severe malaria cases and deaths worldwide. It is also noteworthy that preventing death by malaria is the main goal of the Brazilian National Malaria Control Program (Oliveira-Ferreira et al., 2010).

The present study also revealed a significant decrease in the incidence of infections caused by *P. vivax* and in co-infections (mixed malaria infections) by *P. vivax* and *P. falciparum* ($p<0.0001$). Infections caused by *P. vivax* represent approximately 90% of all malaria cases reported in Brazil (Brazil, 2013), showing that this species greatly contributes to maintaining the malaria epidemic levels in the region.

The reduced incidence of malaria infections noted in this study is due to several factors including the following: Malaria vector control by insecticide spraying; active searches for *Plasmodium*; diagnosis and treatment in loco; the population's awareness of the mode of transmission and of the clinical characteristics of the disease; and especially the use of LLINs because the majority of the bites (and malaria infections) occur indoors and by endophagic mosquitoes, such as *Anopheles darlingi*, which is the main vector of malaria in Brazil and exhibits anthropophilic and endophagic behavior (Deane, 1986). The LLINs, which last for approximately 2 to 3 years (WHO, 2014), protect individuals specifically at this point because some of these bites occur during sleep.

The malaria control programs usually aim to reach at least 80% of the population in risk of catching malaria in a given area, with the use of LLINs (Annual Review, 2008-2009). In the current study, even with only 42% of the surveyed population using LLINs, the results obtained were satisfactory. These data indicate that increasing the use of this device may lead to the effective control of the disease in the municipality, which would be positive not only for health reasons but also aid the economy of the municipality since the high rate of malaria is a potential obstacle to economic development in the area (Williams & Pinto, 2012).

Other studies (Gupta et al., 1999; Langhorne et al., 2008), indicate that the naturally acquired immunity to malaria, the development of which usually requires repeated infections, is important for preventing further worsening of the disease, especially in endemic regions. Therefore, a full coverage of LLINs for all age groups would have a long-term negative impact on the development of natural immunity, especially in children (Doolan et al., 2009; Snow &

Marsh, 2002). One must consider, however, that the losses resulting from the development of naturally acquired immunity in children are lower than the losses resulting from the effects of the disease in malaria-endemic areas where LLINs are not used.

This study demonstrated a decrease in the number of malaria cases in both genders ($p < 0.0001$), with a predominance in males ($p<0.0001$). This finding was demonstrated previously by other studies (Katsuragawa et al., 2009; Vaz & Gonçalves, 2009), and can be explained by the fact that males are more susceptible to malaria infection because of greater contact with the mosquito vector due to their livelihood activities. This hypothesis is reinforced by the fact that the adult age group was more affected by the infection.

This study aimed to investigate the influence of the use of LLINs as a control measure and in combating malaria introduced in the municipality of Pacajá. However, some important variables could not be analyzed thus impairing the discussion and conclusions, namely variables such as: speed and quality of diagnosis, treatment of patients, the number of hospitalizations resulting from infections caused by the disease, besides care with the material distributed. For this reason, further studies are recommended where these parameters are taken into consideration.

This study demonstrated that malaria remains an endemic disease in the municipality of Pacajá-PA. However, in recent years, as occurring in other Amazonian populations, there has been a significant reduction in the incidence of malaria cases when the parameter levels of parasitemia, parasite species, gender and age of the vertebrate host, or simply the total number of infections diagnosed were taken into consideration. Although other factors, such as vector combat by spraying insecticides, rapid and accurate diagnosis of infection and treatment of patients might have influenced this reduced number of malaria infections, the LLINs can be considered a potential disease-control tool because it represents a new variable in combating the disease in the studied population. Regardless of the use of LLINs, adult men are still the most affected because they are responsible for their families' livelihood, which is still based on plant extraction, hunting, and fishing, and these activities increase their exposure to disease vector bites.

Based on the results presented in this study, the expansion of the distribution of LLINs is recommended so that this preventive measure reaches the coverage recommended by other malaria control programs with greater experience in using these devices. In addition, guidance to the population regarding the correct way to use LLINs is required to avoid problems such as the reduced protective effect of the device and the development of resistance to deltamethrin.

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