Original Article

Absence of asymptomatic malaria in pregnant women of Honduras

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Abstract

Background: According to the World Health Organization, 219 million cases of malaria were reported in 2017 worldwide. During the last 8 years, the region of the Americas has experienced an average decrease in the incidence of malaria. Honduras has reported a reduction of 96.3% in the incidence of malaria between 2000 and 2017. Detection of submicroscopic infections is a great challenge for countries with low-endemic settings due to their relevance in the transmission of the parasite to the mosquito. Pregnant women are one of the populations most vulnerable to the complications of malaria and asymptomatic infections are considered as potential reservoirs of infection. The present study estimated the presence of *Plasmodium* asymptomatic infections in pregnant women and their newborns in an area of low endemicity in Honduras.

Methods: Blood samples were collected from 300 asymptomatic mothers, from the umbilical cord of their newborns, and placentas. The DNA was extracted from dried blood spots using the Whatman FTA® purification reagent and the molecular diagnosis of the parasite was performed through two un-nested single tube species-specific PCR tests.

Results: Nine hundred DNA samples successfully amplified the human beta globin partial sequence. None of the samples analysed revealed the presence of the parasite through this methodological approach.

Conclusion: No asymptomatic malaria infections were detected among 300 pregnant women and their children in an area of low endemicity of Honduras. Implementation of more sensitive diagnostic techniques will contribute significantly on preventing transmission in order to eliminate malaria in the Central American sub region.

Key words: Malaria, asymptomatic, pregnancy, Honduras.

Introduction

According to official data from World Health Organization 219 million cases of malaria and 435,000 deaths were reported in 2017 worldwide (1). In the Region of the Americas, 18 countries are still considered endemic, and although the Region has seen an average decrease of 17% in the incidence from 2000 to 2016, there are still national or sub regional foci with disturbing increases in the annual number of malaria cases (e.g. Venezuela, Nicaragua, Peru and Panama).

Nine countries grouped in the sub region of Central America and Hispaniola have one of the most hopeful scenarios regarding the elimination of this disease. Although the nine countries have set the goal of eliminating malaria by 2020, only three countries (Belize, Costa Rica and El Salvador) have reported less than 15 native cases each in 2016 and are projected realistically to eliminate the local transmission by 2020.

In Central America, the most endemic focus of the disease has been La Mosquitia, an ecological region shared by Nicaragua and Honduras. Despite the successes achieved by Nicaragua in reducing the incidence of malaria in the last decade, for the last two years it has reported a significant increase in the incidence and the mortality of malaria.

In contrast, the neighbor country Honduras has reported a continuous decline in malaria incidence of at least 15% per year for the last decade, with only 1,284 cases reported in 2017 compared to 10,513 cases in 2007.

As a result of the Millennium Development Goals for 2015, Honduras has carried out several activities to reduce the malaria burden among the population, such as the distribution of long lasting insecticide bed nets, rapid diagnostic tests in the most inaccessible areas, the improvement in quality and turnaround time of the microscopic diagnosis, timely administration of high quality antimalarial drugs at no cost, drug resistance surveillance, indoor residual spraying, and epidemiological research. As a consequence, the country reports a reduction of 96.3% in the annual incidence of malaria between 2000 and 2017, and those areas historically considered as high and moderate

transmission are now classified as low transmission (parasite prevalence < 10% for 2-9 years) (2).

One of the challenges faced by countries with low transmission settings is the detection of submicroscopic infections, due to the relevance they have in the transmission of the parasite to the mosquito (3, 4). As countries pursue elimination, the implementation of diagnostic tools to survey low-density infections (5, 6) and the active detection (ACD) of asymptomatic hotspots becomes imperative (7, 8). Pregnant women are one of the most vulnerable populations to suffer complications of malaria (severe disease, preterm births and fetal loss) (9, 10), even in regions outside of Africa (11, 12). At the same time, pregnant women are potential reservoir of infection (6). Thus, the goal of this study was to estimate the presence of *Plasmodium spp*. asymptomatic infections in pregnant women and their newborns in an area of low endemicity of Honduras.

Materials and Methods

The study was conducted in the hospital of Tocoa city, located in the department of Colón which was the second department with the highest number of malaria cases in 2017 (19.6%) after Gracias a Dios (25.3%) (1). Malaria is a perennial disease in that region with a moderate peak after the rainy season around the 16 to 20 epidemiological weeks. The recruitment of participants took place between epidemiological weeks 5 and 20, before and during the rainy season. The participants were recruited at antenatal care during the seven days before delivery, where the pregnant women were resting in a facility contiguous to the hospital. After informed consent, and immediately after delivery, blood samples were collected from 300 asymptomatic mothers, from the umbilical cord of their newborns, and from the maternal side of the placentas.

The blood samples were impregnated on Whatman FTA® cards (GE Healthcare, Little Chalfont Buckinghamshire, UK) for further molecular analyses. Microscopy was not performed to search for malaria parasites. The Ethics committee of MEIZ-UNAH reviewed and approved the study (Nº09-2016).

The DNA was extracted from dried blood spots using the Whatman FTA® purification reagent (GE Healthcare, Little Chalfont Buckinghamshire, UK) according to manufacturer´s instructions.

A Harris Uni-Core disposable 2.0-mm punch was used to remove the discs from the centre of dried sample spots. Two hundred µl of FTA Purification Reagent were added to each disc into micro centrifuge tubes. After 5 minutes at room temperature, the buffer was discarded, and this procedure was repeated 2 more times. Three washing steps with 200 µl of TE buffer were carried out. The discs were air-dried and stored at 20ºC until further use for a maximum period of 7 days. In order to verify the DNA quality, the human beta globin gene was amplified according to a previously described protocol with minor modifications (13).

The molecular diagnosis of the parasite was performed through two single tube un-nested PCR tests. Specific primers to *P. falciparum* and *P. vivax* (AL7178 and AL7142; AL7175/AL7074 respectively) were included in two independent PCR reactions for each sample. Amplifications were performed in a 25 µl volume as follow: 2X Taq Master Mix (Promega Corporation®, Madison, WI, USA), 0.4 µM of each primer for *P. falciparum*, and 0.5 µM of each primer for *P. vivax*, and FTA disks as DNA template. Reactions were amplified by an initial denaturation at 95°C for 2 min, 35 cycles of 95°C for 30 sec, 54ºC for 30 sec, and 72°C for 45 sec, with a final extension at 72°C for 5 min. Amplicons were visualized by 1.5% agarose gel electrophoresis with ethidium bromide (14). Positive and negative controls were included in all PCR reactions.

Results and Discussion

Nine hundred DNA samples successfully amplified the human beta globin partial sequence. None of the samples analyzed from mothers (n = 300), umbilical cord (n = 300) or placenta (n = 300), revealed the presence of the parasite through this methodological approach. The positive controls included in each reactions batch amplified properly. No recent history of malaria infections was reported by any of the participants and they all showed absence of malaria-related symptoms at birth. None of the newborns had a body temperature above normal.

These results could indicate that there are no asymptomatic malaria cases among the studied population, in congruence with the significant decline in the number of symptomatic infections in Colón, considered a few years ago as a highly endemic area.

Honduras has shown a remarkable and constant decrease of malaria infections in the last two decades. In 1996, the country reported more than 91,000 malaria infections (15), while in 2017 there were only 1,284 cases (98.6% reduction), and 22 (1.71%) of them were symptomatic pregnant women (1). Based on the recent success of Honduras and the rest of Central American countries in the fight against malaria, the region has set the goal of eliminating the disease by 2020. However, one of the greatest challenges faced by countries willing

to achieve elimination in the short term is the presence of asymptomatic reservoirs among the population (16, 17) because most of these infections remain without detection or treatment (18). One of the most important strategies that characterize a malaria elimination program are those aimed at identifying foci of asymptomatic infections (3, 4, 8, 19).

In this context, pregnant women are a highly vulnerable population, and they have been identified as important reservoirs of infection (6), especially because the detection of malaria during pregnancy is only performed when fever occurs (20), and due to the impossibility of receiving primaquine because of potential toxic effects for the fetus.

Malarial infections during pregnancy are a major public health problem especially in sub-Saharan Africa (21-23), causing relevant complications like maternal anaemia, low birth weight, premature births and stillbirths (24, 25) even without apparent symptoms of the disease. In other endemic regions of the world the burden of malaria during pregnancy is less known (9). Studies conducted in several countries of Latin America suggest very heterogeneous prevalences of malaria among pregnant women depending on the geographical region analyzed, but in any case, they can affect negatively the mother, the placenta, and the newborn (6, 11, 12, 26). Another relevant result of those studies is the significant number of submicroscopic infections among this population, determined by conventional and molecular methods.

Previous studies demonstrate the existence of asymptomatic malaria reservoirs in Honduras. The first report of 1993 analyzed 734 pregnant women in a city with high endemicity, where 9.6% of pregnant women were positive through microscopy. A history of malaria was found in 22.7% of the participants with positive samples, and in only 10.5% of the negatives. The parasite was detected in the peripheral blood of 23.5% of the newborns of parasitized mothers (27). During 2013, a cross-sectional study among 2,554 afebrile school going children from 44 municipalities in the country detected 5 students (0.2%) infected with *Plasmodium vivax* through a nested PCR approach. The five children lived in the same village at Gracias a Dios (28). A year later, 1,899 individuals without malaria symptoms participated in an active case detection (ACD) survey performed also in Gracias a Dios. All samples were analyzed through microscopy and a conventional PCR technique.

The molecular approach was able to detect 1.1% of asymptomatic infections compared to 0.16% detected by microscopy (29). Those studies and our current results show that asymptomatic malaria seems to be low in Honduras with decreasing trend. This downward trend in the number of cases of subclinical or asymptomatic malaria is consistent with the fall in the overall prevalence of malaria in the country and most of the countries in the region.

In this study we used a technique based on single-tube un-nested PCR tests for the detection of the two circulating parasite species (*P. vivax* and *P. falciparum*). This method has demonstrated a sensitivity comparable to the classical nested PCR targeting ribosomal sequences 18S, under conditions of moderate and high parasitaemia (14). However, it is not possible to affirm that the current approach is able to detect very low parasitaemias that are typical of some asymptomatic infections, and this would be the most important limitation of our study. Several publications agree that molecular approaches are more sensitive and convenient for the detection of asymptomatic infections with low parasitaemia compared to microscopy or rapid diagnostic tests (3, 5, 26, 30). Within the molecular methods, there are some that are considered ultra-sensitive, such as RT-PCR or q-PCR (31-34) and that would allow a more sensitive detection of reservoirs for future ACD interventions in a low endemicity setting.

Conclusions

In conclusion, no asymptomatic malaria infections were detected among 300 pregnant women and their children, living in an area of low endemicity of Honduras, however ACD strategies to detect asymptomatic *Plasmodium spp*. reservoirs, and the implementation of more sensitive diagnostic techniques will have the potential to contribute significantly on preventing transmission in order to the elimination of malaria in the Central American sub region.

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