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Asymptomatic plasmodial infection in Colombian pregnant women

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ABSTRACT

Information about asymptomatic plasmodial infection is scarce in the world, and the current antimalarial program goals (control, elimination, and eradication) demand this evidence to be well documented in different populations and malaria transmission settings. This study aimed to measure the prevalence of API in Colombian pregnant women at delivery. A retrospective prevalence survey was used. Women were recruited at hospital obstetric facility in each of the municipalities of Turbo, Necoclí in Antioquia department, and Puerto Libertador in Córdoba department. Malaria infection was tested by thick blood smear (TBS) and real-time quantitative PCR (qPCR). Ninety-six pregnant women at delivery were studied: 95% were asymptomatic (91/96), 45% had asymptomatic plasmodial infection (API) by qPCR (41/91), and only 8% (7/91) had API by microscopy. The prevalence of submicroscopic infections (TBS negative and qPCR positive) was very high, 37% (34/91) in asymptomatic women and 41% (39/96) in total women studied (91 asymptomatic and 5 symptomatic). The prevalence of API in Colombian pregnant women is much higher than which is expected for a country that does not have the level of malaria transmission as Sub-Saharan African countries.

1. Background

Asymptomatic plasmodial infection (API) has been recognized since many decades ago, but only now, it is receiving attention because of the current antimalarial program goals (control, elimination, eradication) (Tietje et al., 2014). There is no a standard definition of API and criteria used in different studies are very diverse (Lindblade et al., 2013). A non-rigid definition of API is presence of erythrocytic plasmodial stages in a person without fever and other symptoms compatible with malaria. Some authors have included parasite density thresholds to define cases of clinical malaria (symptomatic malaria); which means that only a febrile subject with a parasite density above the cutoff is considered a case of symptomatic malaria, while a febrile subject with lower parasite density than the cutoff is classified as “asymptomatic”, because statistically that fever is not attributable to malaria (Smith et al., 1994). This parasite density threshold cutoffs provides a more specific endpoint for vaccine or clinical treatment studies (Lievens et al., 2011), but for burden or impact assessments it should not be used for defining API (Lindblade et al., 2013).

The sensitivity of the diagnostic test used in different studies has impact on the amount of API detected. Microscopy has been the gold standard in malaria research and remains as a point-of-care diagnostic in clinical and epidemiological settings. The rapid diagnostic tests expanded the range of diagnostic options, but their detection limits are only in the range of 100–200 parasites/microliter, compared to around 50 parasites/microliter detected by an expert microscopist. In addition, the development of polymerase chain reaction (PCR)-based tests improved the detection limit for malaria infection to less than 1 parasite/microliter (Wu et al., 2015). Submicroscopic parasite densities (microscopy negative but PCR-based test positive) are common in adults and in chronic infections. It has been reported that microscopy detects only about 54% of all PCR-detectable plasmodial infections, but that percentage has significant variation between studies (Bousema et al., 2014; Okell et al., 2012).

Parasite density is controlled by acquired immunity in infected hosts; therefore, adults are more likely than children to carry submicroscopic infections, and subjects who are repeatedly exposed to malaria have lower parasite densities compared with less-exposed subjects from the same region (Bousema et al., 2014). In addition, there is a general relationship between submicroscopic plasmodial infection (SPI) and API; these two events are different but related because most of SPIs are APIs and vice versa. For example, in Western Thailand 90.2% of *Plasmodium* infections were submicroscopic and asymptomatic (Baum et al., 2016), and in Papua, Indonesia, SPI was significantly more likely in afebrile subjects (adjusted OR = 3.2 [1.49–6.93], p = 0.003) (Pava et al., 2016). In general, studies using molecular detection tools have

Abbreviations: API, asymptomatic plasmodial infection; 95%CI, confidence interval of 95%; PCR, polymerase chain reaction; PSR, plasmodial submicroscopic reservoir; qPCR, real-time quantitative polymerase chain reaction; SPI, submicroscopic plasmodial infection; TBS, thick blood smear

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shown that, in endemic populations, SPI and API are common, whereas symptomatic and microscopically detectable infections comprise only a small fraction of all infections (Bousema et al., 2014).

A number of studies have described the basic characteristics of API in different transmission settings (Alves et al., 2002, 2006; Bousema et al., 2014; Branch et al., 2005; Cerutti et al., 2007; Cucunubá et al., 2008; Cucunubá et al., 2013; Dal-Bianco et al., 2007; de Andrade et al., 1995; Harris et al., 2010; Imperato, 1986; Lindblade et al., 2013; Lynch and Roper, 2011; Roper et al., 1996; Roshanravan et al., 2003; Singh et al., 2014; Steenkeste et al., 2010; Suárez-Mutis et al., 2007; Turki et al., 2015; Zoghi et al., 2012), which include:

- API generally is associated with low parasitemia; a subject with API usually has very few parasites in blood.
- API is associated with the development of clinical immunity-related exposure to *Plasmodium*.
- API is common in adults living in areas with high malaria transmission (Bousema et al., 2014; Dal-Bianco et al., 2007; de Andrade et al., 1995; Imperato, 1986; Roper et al., 1996; Singh et al., 2014; Steenkeste et al., 2010; Suárez-Mutis et al., 2007; Turki et al., 2015); however, in low transmission areas as Solomon Islands and South America there is higher prevalence of API associated with low parasite density (Alves et al., 2002, 2006; Branch et al., 2005; Cerutti et al., 2007; Cucunubá et al., 2008, 2013; Roshanravan et al., 2003). In low transmission settings is difficult to understand the high prevalence of API because those areas have lower exposure to *Plasmodium* than the typical Sub-Saharan Africa high transmission settings (Singh et al., 2014). Lindblade et al. indicated that "without a strong association between transmission intensity and the proportion of infections that are asymptomatic, the population prevalence of asymptomatic malaria infection mirrors the overall transmission level" (Lindblade et al., 2013). Therefore, if transmission intensity increases also increase the percentage of API detected.
- Both *P. vivax* and *P. falciparum* cause API (Cucunubá et al., 2013; Harris et al., 2010; Turki et al., 2015). Both API and SPI are able to infect *Anopheles* vectors and facilitate transmission. Both API and SPI may be due to any plasmodial species (Lindblade et al., 2013). SPI with *P. ovale* is rare, but it happens, while *P. malariae* tends to persist in the bloodstream for decades without causing no or only mild symptoms (Scuracchio et al., 2011).

In general, the available information about API is scarce in the world, and the current antimalarial program goals (control, elimination, and eradication) demand this evidence to be well documented in different populations (children, adults, pregnant women, etc.) and malaria transmission settings. There is evidence that the API is much more common in pregnant women than in non-pregnant women or men. For example, Khan et al., 2014 using a rapid diagnostic test and microscopy, reported the period prevalence of asymptomatic *P. falciparum* infection in pregnant women was 2.3%, compared to 0.5% in non-pregnant women and 0.9% in men (Khan et al., 2014). Moreover, API on pregnant women caused by *P. falciparum* were clearly associated with maternal anaemia (Francine et al., 2016). In general, the APIs are SPIs and vice versa. Those SPIs have been associated with deleterious effects on both pregnant women and their babies; problems as maternal anaemia and low birth weight newborns have a mean frequency of 42% and 16%, respectively, when SPI is present (Arango et al., 2010, 2012; Okell et al., 2009). Therefore, pregnant women are a high risk population group, which must receive priority attention to detect API and SPI. Because of that, this study aimed to measure the prevalence of API in Colombian pregnant women living in the area that produces most cases of malaria each year

2. Material and methods

2.1. Study region

This study was performed among pregnant women that gave birth at one of the obstetric facility of one hospital of municipalities of Turbo (08°05′N,76°44′W), Necoclí (08°25′N; 76°47′W) (Antioquia), and Puerto Libertador (07°54′N; 75°40′W) (Córdoba) during 2008–2013. Other studies have reported results with those women (Agudelo et al., 2013, 2014; Arango et al., 2012, 2013). The three hospitals are public institutions that provide equivalent primary-level healthcare. The economic capacity of the women's families is very low, with poor living conditions (Carmona-Fonseca et al., 2011; Correa Botero et al., 2012). These municipalities are within the malaria transmission region termed Urabá-Altos Sinú and San Jorge-Bajo Cauca, which accounts for 60% of all malaria cases in Colombia. Epidemiologic characteristics of this region are described elsewhere (Carmona-Fonseca, 2003, 2004; Padilla-Rodríguez et al., 2011); briefly, the transmission intensity is low and stable, with no marked fluctuations in the number of malaria cases during the year, and *P. vivax* is reported in approximately 60% of cases. The entomological inoculation rate ranges from 3.5 to 4.8 infective bites per person per year (Naranjo-Diaz et al., 2013). During 2008–2011, the mean annual parasite index (API: malaria cases/1000 inhabitants) in this region was 23.4 (range: 10.2–47.2), while during 2012–2016 the mean API was 8.3 (range: 1.4–25.3) (http://www.ins.gov.co). No national malaria control strategies specific for pregnant women have been implemented and treatment guidelines for malaria in pregnancy include chloroquine for vivax malaria and quinine-clindamycin (first trimester) or artemether-lumefantrine (second and third trimesters) for falciparum malaria (Padilla and Montoya, 2011); the therapeutic efficacy of these schemes is 100% to cure acute malaria attacks (Carmona-Fonseca et al., 2013).

2.2. Study and sample design

A retrospective prevalence survey was used. The sample size was 96 pregnant women at delivery and was calculated based on the formula $n = NZ^2p(1 - p)/[(Ne^2) + (Z^2p(1 - p))]$ (Martínez-Bencardino, 1984) with these parameters:

N = 2600, population; women that gave birth during 2008–2013 in hospitals of Turbo, Necoclí and Puerto Libertador, who were registered in previous studies (Agudelo et al., 2013, 2014; Arango et al., 2012, 2013)

Z = 1.96, confidence level of 95%.

p = 0.25, frequency of the event (asymptomatic plasmodial infection, API). The value of 25% was taken according to 1) Cucunubá et al. in Tierralta, Córdoba, in 2008 with general population (Cucunubá et al., 2008), and 2) other data indicating that API is higher in pregnant women (Arango et al., 2013).

e = 0.1, sampling error, which was set at 10%.

A simple random sampling method without replacement was used to select women who met inclusion criteria (see below) from the total of 2600 women registered in previous studies. All 96 women met inclusion criteria and all of them were analyzed in this study.

2.3. Inclusion criteria

Permanent residence in a malaria-endemic village of Turbo, Necoclí or Puerto Libertador; absence of serious general disease or complicated pregnancy (according to the result of a personal interview); absence of fever (axillary temperature > 37.5 °C) or clinical malaria symptoms at moment of admission to the local hospital obstetric facility (according to the result of a personal interview); absence of use of antimalarial drugs two weeks before admission, availability of samples for malaria diagnosis by thick smear (microscopy) and real-time quantitative PCR (qPCR) (according to the availability of samples in our biobank);