EVALUATION OF CIRCUMSPOROZOITE PROTEIN OF Plasmodium vivax TO ESTIMATE ITS PREVALENCE IN OIAPOQUE, AMAPÁ STATE, BRAZIL, BORDERING FRENCH GUIANA
Margarete Do Socorro Mendonça Gomes, José Luiz Fernandes Vieira, Gustavo Capatti Cassiano, Lise Musset, Eric Legrand, Mathieu Nacher, Vanja Suely Calvosa d’Almeida Couto, Ricardo Luiz Dantas Machado, Álvaro Augusto Ribeiro d’Almeida Couto

To cite this version:

Margarete Do Socorro Mendonça Gomes, José Luiz Fernandes Vieira, Gustavo Capatti Cassiano, Lise Musset, Eric Legrand, et al.. EVALUATION OF CIRCUMSPOROZOITE PROTEIN OF Plasmodium vivax TO ESTIMATE ITS PREVALENCE IN OIAPOQUE, AMAPÁ STATE, BRAZIL, BORDERING FRENCH GUIANA. Revista do Instituto de Medicina Tropical de São Paulo, Instituto de Medicina Tropical, 2016, 58, pp.72. 10.1590/S1678-9946201658072. inserm-01408935

HAL Id: inserm-01408935
https://www.hal.inserm.fr/inserm-01408935
Submitted on 5 Dec 2016

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
BRIEF COMMUNICATION

EVALUATION OF CIRCUMSPOROZOITE PROTEIN OF Plasmodium vivax TO ESTIMATE ITS PREVALENCE IN OIAPOQUE, AMAPÁ STATE, BRAZIL, BORDERING FRENCH GUIANA

Margarete do Socorro Mendonça GOMES(1,2), José Luiz Fernandes VIEIRA(2), Gustavo Capatti CASSIANO(3), Lise MUSSET(4), Eric LEGRAND(4), Mathieu NACHER(5), Vanja Suely Calvosa D’Almeida COUTO(6), Ricardo Luiz Dantas MACHADO(2,3,7) & Álvaro Augusto Ribeiro D’Almeida COUTO(2)

SUMMARY

Malaria is a major health problem for people who live on the border between Brazil and French Guiana. Here we discuss Plasmodium vivax distribution pattern in the town of Oiapoque, Amapá State using the circumsporozoite (CS) gene as a marker. Ninety-one peripheral blood samples from P. vivax patients have been studied. Of these, 64 individuals were from the municipality of Oiapoque (Amapá State, Brazil) and 27 patients from French Guiana (August to December 2011). DNA extraction was performed, and a fragment of the P. vivax CS gene was subsequently analyzed using PCR/RFLP. The VK210 genotype was the most common in both countries (48.36% in Brazil and 14.28% in French Guiana), followed by the P. vivax-like (1.10% in both Brazil and French Guiana) and VK247 (1.10% only in Brazil) in single infections. We were able to detect all three CS genotypes simultaneously in mixed infections. There were no statistically significant differences either regarding infection site or parasitaemia among individuals with different genotypes. These results suggest that the same genotypes circulating in French Guiana are found in the municipality of Oiapoque in Brazil. These findings suggest that there may be a dispersion of parasitic populations occurring between the two countries. Most likely, this distribution is associated with prolonged and/or more complex transmission patterns of these genotypes in Brazil, bordering French Guiana.

KEYWORDS: Plasmodium vivax; Circumsporozoite protein; Brazil–French Guiana border; Genetic marker.

Despite continuous efforts to control malaria, outside Africa, more malaria cases are caused by Plasmodium vivax, resulting in a daunting morbidity and economic burden for many countries across Asia and the Americas, especially in rural areas. Nowadays, P. vivax malaria transmission has decreased during the last decade, although its distribution persists and is heterogeneous in different areas.

Among Amazon countries, Brazil has the highest proportion of malaria cases (56%). In Brazil, gradual decreases from 2006 until 2012 have been observed. Nevertheless, P. vivax now accounts for more than 84% of clinical malaria cases annually reported in the Amazon region. The circumsporozoite protein (CS) of the infective sporozoite is considered to be a major target for the development of recombinant malaria vaccine and, this protein can be evidenced in the process of sporozoite maturation and salivary invasion in the vector as well as in human liver cells. By serological and/or molecular approaches, different authors have evaluated the occurrence of P. vivax variants (VK210, VK247 and P. vivax-like) in endemic areas of the Amazon region. Furthermore, the VK210 and VK247 were detected in An. aquasalis and An. darlingi in endemic areas of Pará State, Brazil.

Border areas between countries are often characterized by intense cross-border population flows. The city of Oiapoque lies on the border between French Guiana and the Brazilian State of Amapá, where there is a well-documented and intense population flux with the French municipality of Saint Georges. In French Guiana malaria is endemic and distributed along the Maroni and Oiapoque rivers, whereas the coastal area bordering the Atlantic Ocean has almost no malaria transmission. A serological study in seven locations of the French Guiana (Cayenne, Camopi, Maripa Soula, Saint Laurent du Maroni, Gran Santi and Sinnamary) suggested that the three variant forms of P. vivax are circulating in these areas.

P. vivax CS genotypes VK210 and VK247 have a worldwide
distribution and have been identified in several studies. However, the
*P. vivax-like* genotype has been only detected in Papua New Guinea,
Brazil, Indonesia and Madagascar.[4,5] In Brazil, the presence of three
variant genotypes was detected in samples obtained from indigenous
populations and other communities of the Amazon region. Furthermore,
there is evidence from Mexico[6] and Brazil[7,8] that VK210 and VK247 have
differential infectivity rates in local vectors. Here we discuss *P. vivax*
distribution pattern in the town of Oiapoque, Amapá State using the CS
gene as a marker.

A subset of 91 patients was analyzed out of 103 individuals previously
evaluated by Gomes et al.[9]. The peripheral blood samples, which had
been kept at -70 °C, were from *P. vivax* carriers who lived in Oiapoque,
Amapá State, a Brazilian malaria-endemic area (Fig. 1). The study took
place from August to December of 2011 and was conducted by the staff
of the Central Public Health Laboratory (LACEN) of the Amapá State.
The patients who were enrolled in this study signed the written informed
consent and fulfilled the following criteria: both genders, aged 16-60
years, they sought medical assistance due to clinical malaria symptoms,
and had a positive malaria diagnosis by thick blood film or molecular
techniques. Of the evaluated patients, for 64 individuals (70.33%) the
infection site was the municipality of Oiapoque and for 27 patients
(29.67%) it was French Guiana.

The DNA was extracted from frozen pellets of infected erythrocytes
using the Easy-DNA™ Kit (Invitrogen, Carlsbad, CA, USA),
according to the manufacturer recommendations. The CS *P. vivax*
genotypes were assessed using PCR/RFLP as previously described
by Cassiano et al.[10]. Briefly, a reaction mix with a final volume of 25
µL containing *P. vivax* DNA (1.5 µL), 1 X PCR buffer (20 mM Tris-
HCl pH 8.4, 50 mM KCl), 1.6 mM MgCl₂, 0.2 mM of each dNTP,
0.2 µM of each primer (5’-AGGCAGAGGACTTGGTGA-3’ and
5’-CCACAGGTTCACACTGCATGG-3’) and 1 U of Taq Platinum.
The reaction was performed in a thermocycler (DNA MasterCycler,
Eppendorf, Madison, WI, USA) as follows: an initial cycle of 94 °C
for 15 min, followed by 30 cycles of 94 °C for 1 min, 58 °C for 1 min
and 72 °C for 1 min, with a final extension at 72 °C for 10 min. As
a positive control, three plasmids were used, containing a gene insert of
the repeated portion of the CSP amplification product from VK210, VK247
and *P. vivax*-like variants (BlueScript, Stratagene, La Jolla, USA). As
the negative control of the reaction, sterile water was used. The RFLP
(Restriction Fragment Length Polymorphism) reaction was performed in
a final volume of 20 µL: 10 U of the restriction enzyme AluI (Invitrogen,
USA), 2 µL of the reaction buffer, 10 µL of the PCR product and 7 µL
of sterile DNase-free water. Reactions were performed in a water bath
at 37 °C overnight.

Analyses were performed using the R statistical software, version
2.4.1 (The R Foundation for Statistical Computing, Vienna, Austria
[http://www.r-project.org]). The distribution of *P. vivax* CSP variants
between the two studied areas was evaluated by the Chi-square test or
the Fisher exact test, and the level of significance was set at p < 0.05.

The distribution of *P. vivax* CS genotypes and the genotypic
frequencies of the 91 blood samples obtained from malaria patients
are summarized in Table 1. The VK210 genotype was the commonest
(62.64%), followed by *P. vivax*-like (22.0%) and VK247 (11.0%) in
single infections. We were unable to detect all three CS genotypes
simultaneously in mixed infections. However, double detections
of VK210 plus VK247 (26.37%) and VK210 plus *P. vivax*-like (7.69%)
were recorded. There were no statistically significant differences for
*P. vivax* CS genotypes and the site of infection (Chi-square, p value =
0.1963). The parasitaemia on the thick blood films ranged from 200 to
36,000 parasites/mm³ (geometric mean + SD: 1,167.86 ± 3.32 parasites/
mm³). The geometric means of parasitaemias were 1,323 parasites/mm³
(1,001- 1,750) for VK210; 1,205 parasites/mm³ (744- 1,951) for the
VK210 + VK247 and 750 parasites/mm³ (295- 1,890) for VK210 + *P.
vivax*-like infections. There were no statistically significant differences
of the geometric mean of parasite density among the different genotypes
detected (Table 1).

Epidemiological and genetic studies performed in malaria endemic
areas of frontier malaria in Brazil could provide valuable information
on parasite transmission and dispersion. The genetic diversity of the CS
gene has been useful in molecular epidemiological studies, understanding
transmission, dynamics and evolutionary relationships[20]. Moreover,
biological and genetic characteristics of the parasite, the host immunity
and local vectors may influence the different patterns of demographic
expansion, which are also modulated by eco-epidemiological conditions.
However, the human effect can become a factor to reduce the risk of
malaria, without necessarily modifying the environment[21].

Parasitic sampling in Oiapoque was performed, and followed a
similar gradual pattern of *P. vivax* CS genotypes observed in others area
of the Brazilian Amazon region. The three genotypes were found as single
and mixed double infections, with VK210 and VK247 more frequently
detected in mixed infections[22]. Interestingly, in French Guiana Volney
et al.[15] performed a seroepidemiological study on malaria and found
positive reactions with all *P. vivax* CS peptides in malaria patients from
the Maroni, Oiapoque and coastal areas. Antibodies against VK210,
VK247 and *P. vivax*-like were used. Our results suggest that this may be a common feature of frontier malaria. Thus, the same genotypes circulating in French Guiana were also found in the municipality of *Oiapoque* in Brazil. Moreover, the detection of VK210 subtypes circulating in the municipality of *Oiapoque* needs to be considered. In our research, some samples diagnosed as VK210 showed a nonspecific fragment in the RFLP gel. Recently, six different VK210 subtypes (VK210a, VK210b, VK210c, VK210d, VK210e the VK210f) were identified in isolates of *P. vivax* from Nicaragua and Mexico. The VK210a subtype was detected in both countries and was the most frequent. VK210f, was the least frequent, and has only been found in Nicaragua. Unfortunately, the sequencing of these samples was not performed, which is a limitation that we acknowledge. Molecular investigations are currently being conducted to understand the role of VK210 subtypes in malaria epidemiology in bordering French Guiana.

This pattern of CS genotypes distribution at the border between Brazil and French Guiana, can bring serious implications to the strategies for effective malaria control. First, approximately 40% of *P. vivax* malaria cases detected in *Oiapoque* are from French Guiana. From another perspective, the *P. vivax* treatment prescription used in Brazil is not the same applied in French Guiana. In Brazil the standard treatment recommended by Brazilian Ministry Health are chloroquine 25 mg/kg for three days (10 mg/kg on day 1 and 7.5 mg/kg on days 2 and 3), plus primaquine 0.50 mg/kg for 7 days. In French Guiana, when the diagnosis is confirmed, the patient has already received an unsupervised three-day treatment with chloroquine (25 mg/Kg). To receive a primaquine prescription, the individual requires a G6PD-deficiency test performed in Cayenne and a nominative temporary use authorization from the State drug authority. Thus, this procedure causes delay in the administration of primaquine. Consequently, approximately half of the population was presented *P. vivax* relapses. Moreover, the periodicity of *vivax* malaria relapses may be explained by the activation of latent hypnozoites by subsequent infections with *P. falciparum*. Evidence from a simultaneous tropical and malaria epidemic suggests that typhoid fever might activate *P. vivax* hypnozoites. However, previous reports suggest that in mixed infections, one *Plasmodium* species may suppress the blood-stage density of another species. Thus, species interactions may cause alterations in the typical clinical manifestation of infections caused by only one species, therefore additional investigations are necessary in order to clarify the role of mixed-infections on disease severity and also in the parasite transmission dynamics, considering that events taking place in French Guiana can influence the transmission and spread of the parasite on the Brazilian side.

In conclusion, a large proportion of all malaria cases in South America occur in the Brazilian Amazon region. The emergence of epidemic and endemic foci is affected by colonization of different areas by different human groups. However, it remains unclear whether parasites are commonly spread from one area to another by migrants or whether they emerge mainly from local endemic populations. The same CS genotypes circulating in French Guiana are found in the municipality of *Oiapoque* in Brazil. These findings suggest that it may occur via the dispersion of parasite populations between the two countries. Most likely, this distribution is associated with prolonged and/or more complex transmission patterns of these genotypes in Brazil, bordering French Guiana. Furthermore, studies based in CSP should consider these effects to obtain a protective vaccine.

**ACKNOWLEDGMENTS**

We thank all the subjects who participated in the study, as well as the health teams and coordination group from the *Oiapoque* District. We thank the following people for assistance in obtaining samples: Manoel do Carmo Barbosa da Cruz and to Valmir Corrêa and Corrêa. We thank Valéria Fraga and Luciana Moran for their technical support.

**CONFLICT OF INTEREST**

The authors declare no conflicts of interest.

**FINANCIAL SUPPORT**

The work reported in this manuscript was funded by the National Scientific and Technological Development in the Research Program for Health System Unique (CNPq PPSUS Public Notice No. 00077/2008/ MCT/CNPq/SETEC, Process No. 35.000.162/2009), and Government of the State of Amapá, and also, Central Public Health Laboratory (LACEN) of Amapá.

**REFERENCES**

1. World Health Organization. World malaria report 2011. [cited 2012 July 24]. Available from: http://www.who.int/malaria/world_malaria_report_2011/en

2. González-Cerón L, Rodriguez MH, Nettel IC, Villarreal C, Kain KC, Hernandez JE. Differential susceptibilities of *Anopheles albimanus* and *Anopheles pseudopunctipennis* to infections with conoidigenous *Plasmodium vivax* variants VK210 and VK247 in southern Mexico. Infect Immun. 1999;67:410-2.
Gomes MSM, Vieira JLF, Cassiano GC, Musset L, Legrand E, Nacher M, Couto VSCD, Machado RLD, Couto AARD. Evaluation of circumsporozoite protein of \textit{Plasmodium vivax} to estimate its prevalence in Oiapoque, Amapá State, Brazil, bordering French Guiana. Rev Inst Med Trop Sao Paulo. 2016;58:72.

3. Stefani A, Dusfour I, Corrêa AP, Cruz MC, Dessay N, Galardo AK, et al. Land cover, land use and malaria in the Amazon: a systematic literature review of studies using remotely sensed data. Malar J. 2013;12:192.

4. Brasil. Ministério da Saúde. Sistema de Informação de Vigilância Epidemiológica: notificação de casos. Malária. [cited 2013 Dec]. Available from: http://portalweb04.saude.gov.br/sivep_malaria/default.asp

5. Herrera S, Bonelo A, Perlaza BL, Fernández OL, Victoria L, Lenis AM, et al. Safety and elicitation of humoral and cellular responses in Colombian malaria-naïve volunteers by a \textit{Plasmodium vivax} circumsporozoite protein-derived synthetic vaccine. Am J Trop Med Hyg. 2005;73 (Suppl 5):3-9.

6. Coppi A, Natarajan R, Pradel G, Bennett BL, James ER, Roggero MA, et al. The malaria circumsporozoite protein has two functional domains, each with distinct roles as sporozoites journey from mosquito to mammalian host. J Exp Med. 2011;208:341-56.

7. Suphavilai C., Looareesuwan S., Good MF. Analysis of circunsporozoite protein–specific immune responses following recent infection with \textit{Plasmodium vivax}. Am J Trop Med Hyg. 2004;70:29-39.

8. Arruda ME, Zimmerman RH, Souza RM, Oliveira-Ferreira J. Prevalence and level of antibodies to the circumsporozoite protein of human malaria parasites in five states of the Amazon region of Brazil. Mem Inst Oswaldo Cruz. 2007;102:367-71.

9. Bonilla JA, Validum L, Cummings R, Palmer CJ. Analysis of the susceptibility to the treatment of malaria by \textit{Plasmodium vivax} in Oiapoque, Brazil, on the border with French Guiana: the importance of control over external factors. Malar J. 2015;14:402.

10. Machado RL, Póvoa MM. Distribution of \textit{Plasmodium vivax} variants (VK210, VK247 and \textit{Pv} vivax-like) in three endemic areas of the Amazon region of Brazil and their correlation with chloroquine treatment. Trans R Soc Trop Med Hyg. 2000;94:377-81.

11. Storti-Melo LM, de Souza-Neiras WC, Cassiano GC, Joazeiro AC, Fontes CJ, Bonini-Domingos CR, et al. \textit{Plasmodium vivax} circumsporozoite variants and Duffy blood group genotypes in the Brazilian Amazon region. Trans R Soc Trop Med Hyg. 2009;103:672-8.

12. da Silva AN, Santos CC, Lacerda RN, Machado RL, Póvoa MM. Susceptibility of \textit{Anopheles aquasalis} and \textit{An. darlingi} to \textit{Plasmodium vivax} VK210 and VK247. Mem Inst Oswaldo Cruz. 2006;101:547-50.

13. Peiter PC, Machado LO, Hiigüez Rojas L. Doenças transmissíveis na faixa de fronteira Amazônica: o caso da malária. In: Miranda AC, Barcellos C, Moreira JC, Monken M, organizadores. Território, ambiente e saúde. Rio de Janeiro: Editora Fiocruz; 2008. p.257-72.

14. Nacher M, Stefani A, Basurko C, Lemonnier D, Djoussou F, Demar M, et al. The burden of \textit{Plasmodium vivax} relapses in an Amerindian village in French Guiana. Malar J. 2013;12:367.

15. Volney B, Pouliquen JF, De Thoisy B, Fandeur T. A sero-epidemiological study of malaria in human and monkey populations in French Guiana. Acta Trop. 2002;82:11-23.

16. Parobek CM, Bailey JA, Hathaway NJ, Socheat D, Rogers MO, Juliano JJ. Differing patterns of selection and geospatial genetic diversity within two leading \textit{Plasmodium vivax} candidate vaccine antigens. PLoS Negl Trop Dis. 2014;8:e2796.

17. Qari SH, Shi YP, Póvoa MM, Alpers MP, Deloron P, Murphy GS, et al. Global occurrence of \textit{Plasmodium vivax}-like human malaria parasite. J Infect Dis. 1993;168:1485-9.

18. Gomes MS, Vieira JL, Machado RL, Nacher M, Stefani A, Musset L, et al. Efficacy in the treatment of malaria by \textit{Plasmodium vivax} in Oiapoque, Brazil, on the border with French Guiana: the importance of control over external factors. Malar J. 2010;9:178.

19. Cassiano GC, Storti-Melo LM, Póvoa MM, Galardo AK, Rossit AR, Machado RL. Development of PCR-RFLP assay for the discrimination of \textit{Plasmodium} species and variants of \textit{P. vivax} (VK210, VK247 and \textit{P. vivax}-like) in Anopheles mosquitoes. Acta Trop. 2011;118:118-22.

20. Souza-Neiras WC, Storti-Melo LM, Cassiano GC, Couto VS, Couto AA, Soares IS, et al. \textit{Plasmodium vivax} circumsporozoite genotypes: a limited variation or new subspecies with major biological consequences? Malar J. 2010;9:178.

21. González-Cerón L., Martínez-Barnetche J, Montero-Solís C, Santillán F, Soto AM, Rodríguez MH, et al. Molecular epidemiology of \textit{Plasmodium vivax} in Latin America: polymorphism and evolutionary relationships of the circumsporozoite gene. Malar J. 2013;12:243.

22. Shanks GD, White NJ. The activation of vivax malaria hypnozoites by infectious diseases. Lancet Infect Dis. 2013;13:900-6.

23. White NJ. Determinants of relapse periodicity in \textit{Plasmodium vivax} malaria. Malar J. 2011;10:297.

24. Lorenzetti A, Fornazari PA, Bonini-Domingos AC, Penhalbel RS, Fugikaha E, Bonini-Domingos CR, et al. Mixed \textit{Plasmodium falciparum} infections and its clinical implications in four areas of the Brazilian Amazon region. Acta Trop. 2008;107:8-12.

25. McKenzie FE, Bossert WH. Multi-species \textit{Plasmodium} infections of humans. J Parasitol. 1999;85:12-8.

26. Machado RL, Póvoa MM, Calvosa VS, Ferreira MU, Rossit AR, dos Santos EJ, et al. Genetic structure of \textit{Plasmodium falciparum} populations in the Brazilian Amazon region. J Infect Dis. 2004;190:1547-55.

Received: 27 March 2015
Accepted: 21 March 2016