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IgG subclasses pattern and high-avidity antibody to the C-terminal region of merozoite surface protein 1 of *Plasmodium vivax* in an unstable hypoendemic region in Iran

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ABSTRACT

The C-terminal region of *Plasmodium vivax* merozoite surface protein 1 (PvMSP-1_{19}) is a leading vaccine candidate for inclusion in a polyvalent malaria vaccine. In the present study, the IgG subclasses profile and the avidity of IgG to PvMSP-1_{19} were evaluated in individuals ($n = 94$) naturally exposed to *P. vivax* parasite in malaria endemic areas in Chabahar districts, Iran. In individuals with patent *P. vivax* malaria, 86.1% was sero-positive to PvMSP-1_{19} and IgG1 (81.9%) was the predominant subclass. In addition, to determine the persistence of specific IgG, IgG1 and IgG3 antibodies to PvMSP-1_{19}, the frequency of antibodies was determined in the infected subjects ($n = 74$) after treatment with standard chloroquine and it was detected that the frequency of responders was significantly reduced to 51.3%, 51% and 16.2%, respectively. The antigen-binding avidity of IgG antibodies to PvMSP-1_{19} was measured in sero-positive sera and the high-avidity of IgG, IgG1 and IgG3 was found in 66.6%, 61% and 47% of the infected subjects with *P. vivax*, respectively. The present result shows that individuals who exposed to vivax malaria in the endemic region in Iran develop antibodies with high-avidity to PvMSP-1_{19}. These results could help to understand the interactions between the host and *P. vivax* parasite in development of MSP-1_{19}-based vaccine.

1. Introduction

Plasmodium vivax is widely distributed and characterized by relapses that generate a significant socio-economic burden (Mendis et al., 2001) in countries such as Brazil, Sri Lanka and Colombia where this species is most prevalent. This parasite re-appeared in Asian countries such as Uzbekistan (Severini et al., 2004), Azerbaijan (Leclerc et al., 2004), Turkey (Zeyrek et al., 2008), north of Iran (Zakeri et al., 2004), and the Republic of Korea (Lim et al., 2000) where eradication efforts had been successful in 1960s. Growing chloroquine and primaquine resistance strains of *P. vivax* in malaria endemic regions (Barat and Bloland, 1997; Nomura et al., 2001) could call for new tools and strategies to combat *P. vivax*. Therefore, to achieve this goal, vaccine development must be considered.

Several antigens from distinct stages of parasite have been identified and considered as potential vaccine targets against parasites. Among the blood stage antigens, the merozoite surface protein 1 (MSP-1) is a leading vaccine candidate for human malaria species *Plasmodium falciparum* and *P. vivax*. MSP-1 is an antigen, abundantly expressed on the surface of mature merozoites (Freeman and Holder, 1983), that is shown to play a role in the invasion of the erythrocyte by parasite (Holder et al., 1992). The C-terminal region of MSP-1 is highly conserved in both *P. falciparum* (Tanabe et al., 1987; Ferreira et al., 2003) and *P. vivax* (Del Portillo et al., 1991; Putaporntip et al., 2002; Zakeri et al., 2006). Antibody to the C-terminal region of this protein (MSP-1_{19}) has been shown to block parasite invasion *in vitro* (Blackman et al., 1994; Chappel and Holder, 1993; Uthaipibull et al., 2001) and this has been validated by several efficacy trials in rodents and primates (Chang et al., 1996; Tian et al., 1997; Yang et al., 1999; Egan et al., 2000). Although intense studies have been performed in PfMSP-1_{19}, less is known about the corresponding protein of *P. vivax* (PvMSP-1_{19}), due in part to the lack of an *in vitro* culture system for this parasite. However, molecular and processing homologies to PfMSP-1 suggest that PvMSP-1_{19} has similar function(s) (Del Portillo et al., 1991; Galinski and Branwell, 1996) and may involve in *P. vivax* merozoites binding to reticulocytes (Rodriguez et al., 2002).

The N- and C-terminal regions of PvMSP-1 were expressed in *Escherichia coli* and tested on Brazilian patients with vivax malaria that most of them were sero-positive for recombinant proteins (Soares et al., 1997). Studies on mice and Saimiri monkeys, immu-

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nized with recombinant PvMSP-1-based antigen, have also shown the protective potential of this antigen (Cunha et al., 2001; Sachdeva et al., 2004; Yang et al., 1999). Further study by Han et al. (2004) revealed that pooled sera from individuals naturally exposed to *P. vivax*, inhibited the binding of PvMSP-1_{19} to human erythrocytes. Importantly, immuno-epidemiological studies have shown a high prevalence of antibodies against PvMSP-1_{19} and an increase in the prevalence of IgG with either age or exposure to *P. vivax* malaria in endemic regions of South America and South East of Asia (Soares et al., 1999; Morais et al., 2005; Lim et al., 2004; Sachdeva et al., 2004; Pitabut et al., 2007; Wickramarachchi et al., 2007). However, only one immuno-epidemiological study of *P. vivax* (using PvMSP-1_{19}) has been conducted in the Middle East (Zeyrek et al., 2008). Therefore, an immuno-epidemiological study in diverse malaria endemic regions with different levels of transmission and human genetic background provides more information to understand the host immune response against *P. vivax* and also it may help to design an effective vaccine against this species.

In Iran, malaria is hypoendemic to mesoendemic with seasonal transmission. In 2007, 16,489 malaria cases were reported from Iran, 85% of the total cases were from south-east that 90% of these cases were *P. vivax* and the rest of them were *P. falciparum* (the Ministry of Health, 2007, unpublished). In this region, there is no record of severe malaria or death due to malaria. Most of the patients are adults and may experience several infections by *P. falciparum* and *P. vivax* with clinical symptoms. Moreover, to date, there is no available published study on the prevalence of asymptomatic carrier in this region. Although high proportions of malaria subjects are due to *P. vivax*, but so far there have not been any immuno-epidemiological studies on vivax malaria in Iran and also its neighboring countries including Afghanistan and Pakistan. Therefore, the present study was designed to characterize naturally acquired antibodies to PvMSP-1_{19} antigen in vivax malaria subject in the hypoendemic region of Iran. In addition, in this study, the avidity of IgG and its subclasses was determined in individuals with patent *P. vivax* infection. The results of this study could help to understand the interactions between the host and *P. vivax* parasite for the development of MSP-1_{19}-based vaccines in most low transmission vivax malaria settings.

2. Materials and methods

2.1. Study area, subjects and blood sample collection

This study was performed in the tropical south-eastern region of Chabahar district in Sistan and Baluchistan, Iran (Zakeri et al., 2002). Transmission is year-round with two peaks, first from May to August with *P. vivax* as the predominant species and the second from October to November when both *P. falciparum* and *P. vivax* infections are recorded. No clinical and sero-epidemiological data are available from this population at the time of writing.

In this investigation, 333 blood samples were obtained from 259 subjects resident in Chabahar district during cross-sectional surveys carried out from May 2005 to 2006. The informed consent was obtained before blood collection from adults or parents or legal guardians of children who were participant in this study. This study was approved by the Ethical Review Committee of Research in Pasteur Institute of Iran. The diagnosis of vivax malaria was made by microscopic examination of blood smears stained with Giemsa-staining in the study areas. From all subjects, 2 and 5 ml venous blood (from children and adults, respectively) was collected for both *P. vivax* DNA detection and serum collection. After transferring to the main laboratory in Tehran, all blood samples were again analyzed for *P. vivax* DNA by nested PCR amplification as described previously (Snounou et al., 1993).

Table 1
Demographic characteristics of the examined groups in this study.

Groups	Sex (%)		Mean age ± SD
	Female	Male	
A (n = 94)	27.7	72.3	26.2 ± 14.9
B (n = 74)	27.1	72.9	21.8 ± 15.4
C (n = 119)	47.9	52.1	25.9 ± 16.13
D (n = 46)	65.2	34.8	31.4 ± 19.85

A = sera samples collected from individuals with patent *P. vivax* infection, B = sera samples collected from 74 out of 94 individuals of group A, three months after treatment, C = sera samples collected from individuals whose last malaria attack due to *P. vivax* occurred six months to 10 years prior to the time of blood sampling, and D = sera samples collected from healthy individuals (negative control).

All 333 blood samples were divided into the following four groups for further study: Group A was composed of 94 sera from individuals with patent *P. vivax* infection. These individuals initiated the treatment with chloroquine after blood collection. The paired blood samples of 74 individuals from group A (74/94) were collected three months after treatment with no sign of re-infection or relapse as group B. The group C was composed of 119 blood samples from individuals whose last malaria attack due to *P. vivax* occurred six months to 10 years ago and they had no re-infection prior to the time of blood sampling. Group D was composed of 46 blood samples from individuals without the history of symptomatic malaria infection as negative control; they were also negative for *Plasmodium* species by standard Giemsa-staining and PCR assay. The demographic information of the examined groups is shown in Table 1.

2.2. DNA preparation

Parasite genomic DNA was prepared by phenol/phenol–chloroform extraction and ethanol precipitation as described previously (Snounou et al., 1993). The DNA was dissolved in 30 μl TE buffer (10 mM Tris–HCl, pH 8.0, 0.1 mM EDTA) and kept at −20 °C until use.

2.3. Cloning and sub-cloning of PvMSP-1_{19}

In our previous study, genetic analysis of PvMSP-1_{19} in the malaria endemic region of Iran revealed that this part of the molecule is highly conserved with no even synonymous mutations (Zakeri et al., 2006). Therefore, randomly, one of the Iranian *P. vivax* isolates (GenBank accession no. AY925098) was used as a template for the amplification of PvMSP-1 fragments corresponding to PvMSP-1_{19} (4837–5181 bp; 115 amino acids with hydrophobic region) with the following primers:

PvpQEF: gCAAggATCCTTATTAACTATgAgCTCCgAgC, BamHI site (underline)
PvpQER: TgTgCCCgggTTAAAgCTCCATgCACAgg, SmaI site (underline)

The gel-purified PCR products were cloned into pGEM-T Easy Vector (Promega, USA) and transformed into *E. coli* DH5α. The transformed clones were selected on the Luria-Bertani (LB) agar medium containing 100 μg/ml ampicilin, 1.5 mM isopropyl-β-D-thiogalactopyranoside (IPTG) and 0.04% X-gal. Positive clones were confirmed by plasmid isolation followed by digestion with EcoRI and the cloned fragments were sequenced. Fragments corresponding to the PvMSP-1_{19} sequence were excised with restriction enzymes (BamHI and SmaI) and ligated to the BamHI–SmaI sites of vector pQE-30 (Qiagen, Germany), which provides a poly-histidine (6-His) tag in N-terminus to facilitate further purification. The ligation mixtures were transformed into competent *E. coli* DH5α cells, and the recombinant clones were selected on ampicillin plates. We confirmed the open reading frame by sequencing and this construct was then used to transform into *E. coli* M15 (pREP4) expression host (Qiagen, Germany). The expression was determined by immuno-