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ORIGINAL ARTICLE

Distribution of Antibodies Specific to the 19-kDa and 33-kDa Fragments of *Plasmodium vivax* Merozoite Surface Protein 1 in Two Pathogenic Strains Infecting Korean Vivax Malaria Patients

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Abstract
Objectives: *Plasmodium vivax* merozoite surface protein 1 (PvMSP1) is the most intensively studied malaria vaccine candidate. Although high antibody response-inducing two C-terminal fragments of PvMSP1 (PvMSP1-19 and PvMSP1-42) are currently being developed as candidate malaria vaccine antigens, their high genetic diversity in various isolates is a major hurdle. The sequence polymorphism of PvMSP1 has been investigated; however, the humoral immune responses induced by different portions of this protein have not been evaluated in Korea.
Methods: Two fragments of PvMSP1 were selected for this study: (1) PvMSP1-19, which is genetically conserved; and (2) PvMSP1-33, which corresponds to a variable portion. For the latter, two representative strains, Sal 1 and Belem, were included. Thus, three recombinant proteins, PvMSP1-19, PvMSP1-33 Sal 1, and PvMSP1-33 Belem, were produced in *Escherichia coli* and then tested by enzyme-linked immunosorbent assays using sera from 221 patients with vivax malaria.
Results: Of the 221 samples, 198, 142, and 106 samples were seropositive for PvMSP1-19, PvMSP1-33 Sal 1, and PvMSP1-33 Belem, respectively. Although 100 samples were simultaneously seropositive for antibodies specific to all the recombinant proteins, 39 and six samples were respectively seropositive for antibodies specific to MSP1-33 Sal 1 and MSP1-33 Belem. Antibodies specific to PvMSP1-19 were the most prevalent.
Conclusion: Monitoring seroprevalence is essential for the selection of promising vaccine candidates as most of the antigenic proteins in *P. vivax* are highly polymorphic.

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1. Introduction

Plasmodium vivax is the most prevalent species that causes malaria in humans [1]. It is endemic in the tropical and subtropical countries of Africa, the Middle East, the South Pacific, Central and South America, and in Asia, including the Republic of Korea (ROK) [2,3]. In recent years, several reports throughout the world have linked *P. vivax* to severe disease and death [4−6]. These findings associated with the emergence of drug-resistant strains have increased concerns regarding this species [7]. Since an effective malaria vaccine capable of inducing robust and long-lasting protection in naturally exposed individuals would be an important tool for malaria control, studies evaluating immune responses against different *P. vivax* vaccine candidates are urgently required.

Proteins expressed on the surface of *P. vivax* merozoites are important candidates for malaria vaccine development. Among these proteins, merozoite surface protein 1 (MSP1) is the most intensively studied. MSP1 is synthesized as a high molecular weight precursor (approximately 200 kDa), which is then processed into several smaller MSPs [8]. During invasion, the C-terminal 42-kDa fragment (MSP1-42) is further processed into 33-kDa (MSP1-33) and 19-kDa (MSP1-19) fragments. Only the MSP1-19 fragment remains on the merozoite surface and is transported into the invaded erythrocytes [9,10]. The C-terminus of MSP1 reportedly induces high antibody responses in hosts, and specific antibodies against this region are known to inhibit merozoite invasion [11,12]. Although both MSP1-19 and MSP1-42 are being considered as potential vaccine candidates, the processing and presentation of these fragments may be problematic due to the large number of disulfide linkages in the two epidermal growth factor-like regions of MSP1-19 [13,14]. In addition, the MSP1-33 fragment, which is the fragment of MSP1-42 without MSP1-19, shows an extensive polymorphism in malaria patient populations [15]. Three representative MSP1 variants of *P. vivax* (PvMSP1)—Belem, Sal-1, and recombinant types—have been observed in the ROK [16,17]. In addition, single-nucleotide polymorphisms have frequently been observed in *P. vivax* isolates from vivax malaria patients [15]. Studies on the MSP1 polymorphism have been performed in the ROK; however, the distribution of strain-specific antibodies has not yet been evaluated [18,19]. In this study, we generated three recombinant proteins of which two correspond to the polymorphic variants of PvMSP1-33 (PvMSP1-33 Sal 1 and PvMSP1-33 Belem) and the other corresponds to the conserved PvMSP1-19. We also evaluated antibody responses to these proteins in individuals infected with *P. vivax* in ROK to determine the frequency and the magnitude of the humoral response against different *P. vivax* vaccine candidate antigens.

2. Materials and methods

2.1. Ethics statement

This study was approved by the research ethics committee of Kyungpook National University (Daegu, Korea). All the participants signed written informed consent forms and agreed to provide 5-mL blood samples.

2.2. Sample collection

The samples were collected at hospitals and health centers throughout the northern region of the ROK, where vivax malaria is endemic in the summer season (June to August). In 2015, 90.4% (619/685) of vivax malaria cases reported in ROK had occurred in this area. Venous blood samples with EDTA were obtained from 221 individuals showing classic symptoms of malaria, who sought treatment at the health facilities mentioned below. The samples were first diagnosed as vivax malaria using a rapid diagnostic test kit (NanoSign Malaria P.f/P.v; Bioland, Seoul, Korea) at a hospital or health center. After blood collection and diagnosis, all the patients were treated with chloroquine. First of all, 600 mg chloroquine was administered, and then three more doses of 300 mg chloroquine were administered at 6 hours, 24 hours, and 48 hours after the first dose.

The blood samples were centrifuged at 1,500*g* for 15 minutes to obtain plasma for further studies. The plasma samples were transported on ice to a laboratory in the Department of Parasitology and Tropical Medicine at Kyungpook National University and were stored at −70°C until required. The 221 blood samples from vivax malaria patients consisted of 140 and 81 samples collected in 2012 and 2013, respectively. All the samples were collected from symptomatic vivax malaria patients who had not been previously infected with *P. vivax*. The mean age of the patients at sampling in 2012 and 2013 was 43.2 years and 36.4 years, respectively. Of all patients from whom samples were collected from in 2012 and 2013, 40.0% (56/140) and 30.9% (25/81) were from men, respectively. In most samples, the *P. vivax* parasite was not observed using microscopy due to low parasitemia, even though the rapid diagnostic test and nested-polymerase chain reaction (PCR) were positive. Samples were also obtained from 30 healthy volunteers who resided in nonendemic areas (southern parts) of the ROK and had not traveled to vivax malaria endemic areas.

2.3. Microscopic examination and nested-PCR for vivax malaria diagnosis

All the blood samples were examined using microscopic analysis of Giemsa-stained thick and thin blood films and using nested-PCR, targeting the 18S ribosomal RNA as described previously [20,21]. Briefly, 5 μL of extracted DNA was used as the template in a primary