Alternative Malaria Diagnostic Tools: Evaluation of Plasmodium falciparum Detection along Thailand’s Border by Loop-Mediated Isothermal Amplification (LAMP) and Immunochromatographic Test (ICT)

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Abstract

Introduction: Thailand has a national goal to eliminate malaria from 80 percent of the country by 2020. However, malaria-endemic areas still exist along the forested Thailand’s borders. An accurate and prompt diagnosis is the key to effective disease management. This aim of this study was to evaluate the application of LAMP as an alternative tool for falciparum malaria diagnosis of clinical suspicion of plasmodium infection in the border area, compared to those of ICT and microscopy using nested PCR as the reference detection method.

Materials and Methods: One hundred and four of blood samples were obtained from patients who were diagnosed malaria positive by clinical diagnosis or subjected to suffering from malaria as observed from clinical history. The primer set used for LAMP was designed on the basis of nucleotide sequence of 18S rRNA plasmodium gene and the ICT test was performed with the parasite lactate dehydrogenase (pLDH) antigen-based lateral flow test.

Results: The LAMP assay has the highest agreement with the reference method (99.04%, k=0.98) with a sensitivity (95% CI) of 98.59% (95.85-100.00) comparable to ICT and microscopy. Moreover, LAMP showed specificity 100% compared with 100% of ICT and 96.97% (91.12-100.00%) microscopy. Negative predictive value of LAMP and ICT were 97.06% and 82.80% respectively.

Conclusion: LAMP is useful and more reliable for specific diagnosis in border regions where malaria is endemic but individuals are asymptomatic and hence that LAMP could be the preferred method in resource-limited laboratories to progress towards malaria elimination in Thailand.

Keywords: Loop-mediated isothermal amplification; Immunochromatographic test; Nested polymerase chain reaction

Introduction

Malaria is an endemic disease which is caused by Plasmodium parasites. There are five parasite species that cause malaria in humans; Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae, Plasmodium ovale and Plasmodium knowlesi [1]. P. falciparum and P. vivax are the most common but P. falciparum is the most deadly. An accurate and prompt diagnosis is the most common and needed to deploy the impact of effective treatment especially for potential fatal cases of P. falciparum [2,3]. Thailand has experienced a 70 percent decrease in reported malaria cases between 2000 and 2011 [4]. Central Thailand has been malaria-free for more than three decades [5]. Therefore, Thailand has a national goal to eliminate malaria from 80 percent of the country by 2020 [6]. However, malaria-endemic areas still exist along the forested Thailand’s borders with Myanmar to the west and Cambodia to the east [5]. Several major outbreaks occurred along Thailand’s borders [7]. Moreover, the border area has been the epicentre of multidrug resistant P. falciparum [8]. The patient’s signs and symptoms of malaria are nonspecific and variable [2,9]. Accordingly, differentiation of clinical diagnosis is sometimes difficult and confirmatory diagnosis using laboratory technologies is needed. An accurate parasite-based diagnosis of malaria is essential for proper treatment of individual patients [10].

Conventional laboratory diagnosis has relied on microscopy. It is an accurate tool but requires well-trained staff. Interpretation of smear requires expertise. Recently, the Immunochromatographic test (ICT) have become available, which are based on the recognition of Plasmodium antigen in the blood circulation of patients. The test is rapid and simple. Following WHO recommendation, ICT were used as rapid diagnosis test for malaria infection [11]. More recently, the interpretation of malaria diagnosis and epidemiology has changed by using molecular tools, for instance by revealing grand reservoirs of asymptomatic infection and, by detecting distribution of Plasmodium spp. infection. According to DNA amplification, nested polymerase chain reaction (nested PCR) method targeting 18S rRNA gene, all species could be identified [12,13]. However, a major drawback of the molecular detection tool is its cost, requiring PCR machine and well-trained staff. The loop-mediated isothermal amplification (LAMP) is a most recently developed molecular technique that overcome disadvantage of PCR by being simpler and faster. The cost of the technique can be reduced by using a water bath or a heat block instead of PCR [14-16].

The aim of this study was to evaluate the application of LAMP as an alternative tool for falciparum malaria diagnosis of clinical suspicion of plasmodium infection in the Thailand border area, compared to those of ICT using nested PCR as the reference detection method.

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