# RESEARCH NOTE

# RAPID IMMUNOCHROMATOGRAPHY-BASED DETECTION OF MIXED-SPECIES MALARIA INFECTION IN PAKISTAN

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Abstract. We report the identification of mixed *Plasmodium* infections in four recent patients with malaria clinically refractory to empiric chloroquine therapy using the rapid antigen detection kit, NOW® ICT Malaria Pf/Pv. A rapid *in vitro* immunodiagnostic test, the NOW® ICT Malaria Pf/Pv test kit was used for the detection of circulating *Plasmodium falciparum* (Pf) and *Plasmodium vivax* (Pv) antigens in whole blood. Peripheral blood microscopy confirmed mixed-species infection in all the cases. Thick and thin peripheral blood films were made and stained with Giemsa stain and examined by both hospital laboratory staff and an experienced parasitologist who was blinded to the results of the rapid malarial antigen tests. Four recent patients (all male; mean age, 24 years) with mixed malarial infection were identified. All the subjects were males working for an oil company in a coastal area of Pakistan, and all had been diagnosed presumptively with malaria based on clinical grounds (without microbiologic confirmation), and were treated empirically with chloroquine without clinical response. Semiquantitative malaria counts via microscopy were as follows: *P. vivax*, scanty (2 patients) and moderate (2 patients); for *P. falciparum* – scanty (1 patient), moderate (2 patients), and heavy (1 patient). The present case series, although limited by the small number of patients with proven mixed *P. falciparum-P. vivax* infection, highlights the usefulness of the rapid antigen test in a highly malarious region of Pakistan where chloroquine resistance is prevalent. Although there was full concordance between the results of blood smear microscopy and rapid antigen testing, these techniques are potentially most useful when there is a discrepancy with microscopy findings. Accurate and rapid diagnosis of parasites, particularly in cases of mixed *P. falciparum* and *P. vivax* infection, is of immense importance for individual patient management and in reducing the burden of disease, especially in regions of chloroquine resistance.

## INTRODUCTION

Malaria is a disease of enormous global significance and impact, causing approximately three million deaths worldwide each year, mainly in developing countries (WHO, 2000). Rapid and accurate diagnosis of malaria is essential if effective therapy is to reduce morbidity and mortality associated with the disease. Microscopic examination of Giemsa-stained peripheral blood smears has been the mainstay of malaria diagnosis for decades. More recently, there has been tremendous progress in the development of a number of rapid and specific antigen diagnostic tests to identify patients infected with malaria (Piper *et al*, 1999; Stephans *et al*, 1999; Smego and Beg, 2000).

The occurrence of mixed species malaria infections is a concern in many parts of the world (May *et al*, 1999; Pinto *et al*, 2000). Infections with three different species of *Plasmodium* have been reported (May *et al*, 1999). With expanding global resistance to chloroquine, including Pakistan and Afghanistan (Shah *et al*, 1997; Rab *et al*, 2001), it is prudent to emphasize the importance of early detection of mixed-species malaria infections and appropriate treatment of chloroquine-resistant *P. falciparum*, and occasionally *P. vivax*, infections. We herein report four recent patients from a coastal region of Pakistan who had concomitant *P. falciparum* and *P. vivax* infections that were diagnosed by the use

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of a rapid, immunochromatography-based antigen detection method.

## MATERIALS AND METHODS

This retrospective case series study was conducted at The Aga Khan University Hospital (AKUH), a 622-bed tertiary care academic hospital serving the residents of Karachi, Pakistan, and surrounding Sindh Province. Microscopy logbooks of the Clinical Parasitology Laboratory were scanned in order to identify recent patients with mixed malarial infection. In addition, logbooks maintained on rapid malarial antigen testing were also examined.

For rapid malaria antigen detection, the NOW® ICT Malaria Pf/Pv test kit (Abbott Diagnostics, Chicago, IL, USA) was used. The technique is a rapid *in vitro* immunodiagnostic test for the detection of circulating *Plasmodium falciparum* (Pf) and *Plasmodium vivax* (Pv) antigens in whole blood. The test uses two antibodies, which have been immobilized on a test strip. One antibody is specific for the histidine-rich protein 2 antigen of *P. falciparum* (Pf HRP2). The other antibody is specific for a malarial antigen, which is common to both *P. falciparum* and *P. vivax* species. For testing, 5 ml of venous blood was drawn in glass tubes treated with ethylenediamene tetraacetic acid (EDTA). A few drops of blood were applied to the kit along with a reagent. Thick and thin peripheral blood films were made and stained with Giemsa stain and examined by both hospital laboratory staff and an experienced parasitologist (one of the authors). The parasitologist was blinded to the results of the rapid malarial antigen tests. Peripheral blood microscopy was considered the diagnostic "gold standard" and confirmed mixed-species infection in all four cases.

## RESULTS

Four recent patients with mixed malarial infection were identified. All subjects were males working for an oil company in a coastal area of Pakistan, and all had been diagnosed presumptively with malaria based on clinical grounds (without microbiologic confirmation), and were treated empirically with chloroquine without clinical response. The clinical and laboratory characteristics of the patients upon presentation to AKUH are shown in Table 1.

## DISCUSSION

The pitfalls in the management of patients with mixed-species *Plasmodium* infections have been well described (Hess *et al*, 1993). Initial identification of non-falciparum malaria species only by peripheral blood smear may lead to improper treatment with chloroquine alone. The present study, although limited by the small number of patients with proven mixed *P. falciparum-P. vivax* infection, highlights the usefulness of a rapid antigen test in a highly malarious region of Pakistan where chloroquine resistance is prevalent. In our series, there was full concordance between the results of blood smear microscopy and rapid antigen testing. However, rapid ma-

Table 1
Clinical and laboratory characteristics of patients with mixed-species malaria infection.

| Case | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Age (yrs) | 22 | 28 | 24 | 23 |
| Sex | Male | Male | Male | Male |
| Presenting complaint | Fever and chills | Fever and chills | Fever and chills | Fever and chills |
| Physical findings | None | Jaundice, splenomegaly | None | None |
| Hemoglobin (g/l) | 15.4 | 10.2 | 13.2 | 17 |
| Platelets (per $mm^3$) | 43,000 | 28,000 | 78,000 | 75,000 |
| Serum creatinine (mg/dl) | 1.2 | 4.9 | 0.9 | 1.1 |
| Malaria species and count via microscopy | Pf scanty<br>Pv scanty | Pf heavy<br>Pv moderate | Pf moderate<br>Pv scanty | Pf moderate<br>Pv moderate |
| Malaria antigen test result | Mixed | Mixed | Mixed | Mixed |
| Complication | Acute renal failure | None | None | None |

larial antigen tests are potentially most useful when there is discrepancy with microscopy findings. Sensitivities and specificities of between 84-100% and 89-100%, respectively, have been reported for various newer diagnostic methods (Jelinek *et al*, 1999; Mills *et al*, 1999; Mishra *et al*, 1999; Tjitra *et al*, 1999; Ricci *et al*, 2000; Smego and Beg, 2000).

Of 531 patients with microscopically-confirmed malaria at our tertiary care institution, we recently found the prevalence of mixed malarial infection to be 1.3% (Beg *et al*, 2004)), a finding similar to that reported in Thailand and Lao PRD with prevalences of <2% and <1%, respectively (Mayfong *et al*, 2004). Using microscopy alone may under-diagnose mixed malaria infections; rapid testing can enhance diagnostic yields, but these assays are not used routinely.

Where chloroquine resistance exists, the presence of mixed malaria and the inappropriate use of chloroquine exerts pressure on the host-parasite-vector relationship. Chloroquine-sensitive *P. vivax* is eliminated, while resistant *P. falciparum* persists and extends its domain. In the few regions where *P. falciparum* remains susceptible to chloroquine, timely detection of mixed malaria infection may not be as critical for successful individual patient management. However, in most of the world, the prevalence of chloroquine-resistant falciparum malaria demands accurate and early detection of this species so that appropriate antimalarial agents can be added to the initial treatment regimens. Pakistan is a known area of chloroquine resistance confirmed by *in vitro* susceptibility testing (Beg *et al*, 2002). Similarly, in geographic areas where evolving resistance to chloroquine is seen among isolates of *P. vivax*, non-chloroquine treatment alternatives must be selected.

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