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; Stephens 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...
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>
<thead>
<tr>
<th>Case</th>
<th>Clinical and laboratory characteristics of patients with mixed-species malaria infection.</th>
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<tbody>
<tr>
<td>Age (yrs)</td>
<td>22</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
</tr>
<tr>
<td>Presenting complaint</td>
<td>Fever and chills</td>
</tr>
<tr>
<td>Physical findings</td>
<td>None</td>
</tr>
<tr>
<td>Hemoglobin (g/l)</td>
<td>15.4</td>
</tr>
<tr>
<td>Platelets (per mm³)</td>
<td>43,000</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>1.2</td>
</tr>
<tr>
<td>Malaria species and count via microscopy</td>
<td>Pf scanty</td>
</tr>
<tr>
<td>Malaria antigen test result</td>
<td>Mixed</td>
</tr>
<tr>
<td>Complication</td>
<td>Acute renal failure</td>
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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.

REFERENCES


