An investigation on the prevalence and efficiency of immunochromatographic testing in suspected malarial patients of Rawalpindi and Islamabad, Pakistan

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Background/aim: The prevalences of Plasmodium falciparum and Plasmodium vivax are increasing rapidly in Pakistan, but recent data on the epidemiology of malaria are not properly reported with scarce diagnostic methods for quick diagnosis. This study was designed to determine the current prevalence and distribution of Plasmodium species in the vicinity of Rawalpindi and Islamabad and report on the validity of the immunochromatographic test (ICT) in diagnosing malarial infections.

Materials and methods: A total of 1500 blood samples obtained from a local hospital were screened during the course of this study via microscopic examination and ICT.

Results: It was seen that malaria was highly endemic in this region. Both P. vivax and P. falciparum were prevalent in all age groups with high seasonal variations, showing a summer peak for P. vivax and a winter peak for P. falciparum. In a comparative study of the diagnostic methods it was observed that the ICT is 95% sensitive and 100% specific for both P. falciparum and P. vivax, while microscopic study was 100% sensitive and 96.8% specific.

Conclusion: Epidemiological study of the malarial parasites showed that majority of the patients were from Rawalpindi as compared to Islamabad and that P. vivax was the dominant cause of malarial infection.

Key words: Malaria, immunochromatographic test, Plasmodium falciparum, Plasmodium vivax

1. Introduction
The information generated from the completion of the genome sequence of Plasmodium falciparum in 2002 helped revolutionize molecular biological research on malarial parasites. Malaria is among the oldest documented diseases of humans and even today organisms in the genus Plasmodium infect more people than do the vectors of any other infectious disease (1). Temporal variation of malaria cases shows a significant positive association with meteorological variables including average monthly rainfall and temperature (2). According to the World Health Organization (WHO), approximately 135 to 287 million cases and 473,000 to 789,000 deaths due to malaria were reported in 2012 (3).

Four species of malaria parasites, Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, and Plasmodium malariae, are known to infect humans (4). Among these, P. vivax puts billions of the world’s population at risk of infection due to greater survival ability in challenging environments (5–7). Malarial infection is one of the gravest problems prevailing in Pakistan, where population explosion, low per capita income, poor health facilities, poor nutrition in joint family systems, lack of education, and unhygienic communities contribute towards an increase in the incidence of malarial infections. According to the WHO, 60% of the Pakistani population lives in endemic malarial regions (3). After eradication efforts in the 1960s, malaria surged back to an epidemic level in the 1970s. An upward trend in the prevalence of malaria was partially attributed to floods that affected approximately 20 million people in over 60 districts (8). Despite a well-established malaria control program, 500,000 malaria infections and 50,000 malaria-attributable deaths occur each year in Pakistan (9), with approximately 37% of cases occurring in regions along the borders with Afghanistan and Iran (10).

Detection of mixed infections can be difficult due to varying levels of parasitemia, low organism density, and
confusion among various morphological criteria for identification to the species level. Using polymerase chain reaction (PCR) methods, a higher detection rate of chronic and mixed malarial species is possible (11). Microscopic examination of thin and thick blood smears remains the gold standard to diagnose malaria; however, quick and convenient rapid diagnostic tests are effective but require improved quality control and are also costly. Other techniques including serological tests and molecular-biological techniques also give good results in less time (12). The reported decrease of sensitivity at medium and low levels of parasitemia of the immunochromatographic test (ICT) has cast doubts as to whether the ICT can replace properly performed microscopy diagnosis at present (13), despite the fact that the ICT is much more rapid and simpler than the thick blood smear examination method for the diagnosis of P. falciparum malaria and can be applied in outpatient clinics in endemic areas (13,14). In the current study, an attempt was made to check the validity of the ICT for the diagnosis of P. falciparum and P. vivax and to compare the prevalence of these human malarial infections among the inhabitants of Rawalpindi and Islamabad.

2. Materials and methods
To carry out this research, blood samples of patients of all age groups attending Benazir Bhutto Hospital-Rawalpindi, Holy Family Hospital-Rawalpindi, and Pakistan Institute of Medical Sciences-Islamabad were screened for the presence of malarial parasites. All these patients showed general signs and symptoms related to malaria, such as fever, headache, rigors, sweating, nausea, vomiting, and anemia.

2.1. Blood sample collection
The study’s time period spanned 1 year, from September 2012 to August 2013. In total 1500 blood samples were collected from patients visiting the different hospitals of Rawalpindi and Islamabad, after fulfilling all ethical considerations pertaining to permissions and consent, and the samples were immediately transported to the Parasitology Laboratory at PMAS-Arid Agriculture University, Rawalpindi, for characterization of Plasmodium spp. Furthermore, demographic details of each patient were recorded on prescribed questionnaires. A pilot study was conducted prior to the selection of samples so as to validate the questionnaire.

2.1.1. Preparation of blood films
Thick blood films were prepared by taking 3 or 4 drops of blood on approximately 1.25 cm from the end of glass slides. These drops were spread with the corner of another slide by a circular motion over an area of approximately 3.8 cm in diameter. Blood was distributed as evenly as possible. Stirring was continued for 30 s to prevent formation of fibrin strands that may obscure the parasite after staining. The film was allowed to air-dry in a dust-free area. Prior to staining, the thick blood film was flooded with distilled water to remove hemoglobin.

2.1.2. Processing of blood films
Thick and thin blood films were made on the same slide. The thin film was fixed with xylene and the whole of the slide was stained for 30 min with 10% Giemsa stain. The thick film was used to see parasites and their count, but species diagnosis was confirmed with the help of the thin film.

2.2. Parasitological examination
Thick smears were examined for 10 min each under 100× magnification. When a thick smear was found positive for malarial parasite, the thin smear was then examined for specific identification.

2.2.1. Immunochromatographic testing
The ICT (NOW ICT Malaria Pf/Pv. Test, Binax Inc., Portland, ME, USA) is based on an antigen capture assay. The specific antigen for P. falciparum is a histidine-rich protein-II (Pf-HRP-II) and plasmodium lactate dehydrogenase (PLDH) for all human malaria parasites. Detection of Pf-HRP-II or PLDH antigens is performed by IgG monoclonal antibodies, which are prepared against these antigens (15). The test card was opened and placed on a smooth surface; the blood sample was taken by capillary tube from the patient’s pricked finger and was put on the purple area of the sample pad of the dipstick strip located on the right side of the card. Once the purple pad was saturated with blood sample, two drops of reagent A were poured immediately below the purple pad and four drops on a cleaning pad located on the top of the left side of the card. After running up the lysed blood to a limit line on the strip, the card was closed. The result was then read through a viewing window, 3–5 min after the color of the blood had almost entirely cleared. The test was considered positive when lines appeared in front of P. falciparum or P. vivax and negative when only a control line was observed. If no line or only the test line appeared, the result was considered invalid.

2.3. Statistical analysis
The data were arranged in appropriate groups and analyzed by applying the chi-square, proportion, and double proportion tests using SPSS 20. Commonly used performance indices, such as sensitivity and specificity, were computed for each of the rapid diagnostic tests, and the indices included sensitivity [true positive / (true positive + false negative)] and specificity [true negative / (true negative + false positive)].

3. Results
In the present study it was observed that the ICT is 95% sensitive and 100% specific for both P. falciparum and P.
vivax, while microscopic study was 100% sensitive and 96.8% specific (Figures 1A and 1B).

Among 1500 patients, 38% were found to be malaria-positive when they were tested by microscopy and ICT, as shown in Figure 1A. By applying the double proportion test \((Z = -0.406)\), it was observed that the prevalence of malarial infection varies in the inhabitants of Rawalpindi and Islamabad (Figure 2). Among 570 malaria-positive patients, 315 (55.3%) were from Rawalpindi and 44.73% were from Islamabad.

The prevalence of \(P. \text{vivax}\) was the same in both Rawalpindi and Islamabad at 50%, whereas \(P. \text{falciparum}\) was more prevalent in Rawalpindi (62.5%) as compared to Islamabad (37.5%). The present findings revealed that the rate of infection was highest from May to August (49.7%), followed by September–December (39.58%), and lowest in January–April (12.5%) (Figure 3). The parasite prevalence during May–August was 47.9%, and 65.22% of infections were caused by \(P. \text{vivax}\). Similarly, \(P. \text{falciparum}\) was highest at 56% during September–December (Figure 3).

By applying the chi-square test \((\chi^2 = 8.0336)\), some relationships were observed between age and disease. The incidence of malaria recorded in the age group of 1–10 years was 26.3%, but a higher incidence was reported in the age group of 11-20 years (36.8%). A decline was found in infection rates (15.79%) in those 21–30 years old and above 30 years old (21.1%) (Table). \(P. \text{falciparum}\) infections were more common in male patients (62.5%) as compared to female patients (37.5%), while a negligible difference was observed between male (45.5%) and female (54.5%) patients infected with \(P. \text{vivax}\).

Malaria due to \(P. \text{falciparum}\) was more common in patients aged 1–20, whereas infection due to \(P. \text{vivax}\) persisted in all ages. Hence, age is an important factor that affects the rate of infection and individuals below 20 years of age were more commonly affected because they have more exposure to those conditions in which malaria is
acquired and transmitted. The infection rate was highest in students (42%) of different ages, followed by laborers (24%) with low social class status. Malarial infections in other patients belonging to different occupations were relatively low (Table).

### 4. Discussion

The current study found the ICT to be 95% sensitive and 100% specific for both *P. falciparum* and *P. vivax* as compared to microscopic study, which was 100% sensitive and 96.8% specific. These results are in accordance with the findings of previous researchers (16–23). The widely reported accuracy of the ICT in diagnosing both *P. falciparum* and *P. vivax*, with the added advantage of it being a rapid test, makes the value of the ICT in making on-the-spot diagnoses significant.

Malarial transmission is strongly associated with features of the habitat. The disease is focused around specific mosquito breeding sites and can normally be transmitted only within certain distances from them: in Africa these are typically between a few hundred meters and a kilometer, rarely exceeding 2–3 km (24). The patterns of malaria morbidity and mortality can vary with the level of malaria transmission in a given area (25). According to Shomakhov (26), urban and rural residents were positive at rates of 27.9% and 72.1%, respectively, when screened for malaria infections (27).

The results of this current research, depicting a higher incidence of malaria for the age group of 11–20 years old as compared to other age groups, are in accordance with the work conducted by Giha et al. (32), who found that malaria risk was highest in individuals of 5–19 years old. Strickland et al. (33) also reported that infection rates in Punjab, Pakistan, are much higher in children than in adults, for both *P. falciparum* and *P. vivax*. The incidence of severe malaria was reported to be 5–7 times higher in children aged 1–4 years in Burkina Faso (34,35) (Table).

Previously Coleman et al. (22) observed that males (54.6%) were more affected than females (45.6%). Similar

### Table. Demographic distribution of malarial species among 570 positive patients.

<table>
<thead>
<tr>
<th></th>
<th><em>P. falciparum</em></th>
<th><em>P. vivax</em></th>
<th>Total*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>150</td>
<td>150</td>
<td>300</td>
</tr>
<tr>
<td>Female</td>
<td>90</td>
<td>180</td>
<td>270</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–10</td>
<td>75</td>
<td>75</td>
<td>150</td>
</tr>
<tr>
<td>11–20</td>
<td>120</td>
<td>99</td>
<td>219</td>
</tr>
<tr>
<td>20–30</td>
<td>15</td>
<td>66</td>
<td>81</td>
</tr>
<tr>
<td>&gt;30</td>
<td>30</td>
<td>90</td>
<td>120</td>
</tr>
<tr>
<td><strong>Occupation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Govt.</td>
<td>11</td>
<td>10</td>
<td>21</td>
</tr>
<tr>
<td>Laborer</td>
<td>30</td>
<td>65</td>
<td>95</td>
</tr>
<tr>
<td>Student</td>
<td>105</td>
<td>135</td>
<td>240</td>
</tr>
<tr>
<td>Businessman</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Household work</td>
<td>45</td>
<td>58</td>
<td>103</td>
</tr>
<tr>
<td>Others</td>
<td>49</td>
<td>60</td>
<td>109</td>
</tr>
</tbody>
</table>

*: 570 positive patients.
results were also reported by Gehlawat et al. (36) and Singh et al. (37).

Malarial infections are reported to be related to occupation as some work and environmental conditions may prove to be conducive to the spread of malaria (38). The findings of the current study highlight the fact that most of the patients suffering from malaria were students, which pertains more to their age group than their occupation. All other occupations had low levels of malaria prevalence. It was concluded that although the infection of malaria persists throughout the year, the species of *Plasmodium* vary greatly during different months of the year. The ICT was found to be adequately sensitive as well as specific in diagnosing infections. The rapid diagnosis achieved via the use of the ICT makes it a viable option for making on-the-spot diagnoses rather than waiting for longer periods of time for laboratory test results. Malaria is found to be more common in males, in younger age groups (between 11 and 20 years old), and in rural areas of the country. Occupation also had an impact on the prevalence of malaria in Pakistan.

**Acknowledgments**

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**References**


