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Diagnosis of Plasmodium Falciparum Infection Using Immuno-Chromatography Test in Blood and Urine of Sudanese People in Elgadaraf State

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ABSTRACT

Malaria *Plasmodium falciparum* rapid card test (QDx rapid malaria test) is an immunochromatographic test that detects the presence of malaria antigen (HRP-2) in blood samples. These malaria antigens are also released in the urine. This study was conducted to determine the sensitivity, specificity of the QDx Rapid Malaria test for the diagnosis of malaria in blood and urine. Blood and urine specimens were obtained from 90 malaria blood smear-positive cases (test samples) and 90 malaria negative cases (controls). The urine and blood specimen was collected for QDx rapid malaria test. Using microscopy as the gold standard, the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the QDx rapid malaria test for urine and blood were calculated. The sensitivity of QDx rapid malaria test for malaria parasite detection was 92.2% for blood and 7.7% for urine. Rapid malaria test processed with urine may be unuseful in detecting falciparum malaria antigens.

Keywords: Rapid malaria test, HRP-2, Urine, Sensitivity and Specificity.
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INTRODUCTION

Malaria is one of the most common parasitic diseases and a major health problem, worldwide infecting 200 million and killing about 2 million people each year (Cattani et al., 1993). Rapid diagnosis and early treatment of clinical cases are central to the reduction of malaria morbidity (Smegoraj and Beg, 2000). The two diagnostic approaches currently used are clinical and microscopic examination. Clinical diagnosis of malaria alone is unreliable and should be confirmed by laboratory tests (Iqbal et al., 2002). Currently, the vast majority of cases in the world are detected by light microscopy of stained blood smears which remains the gold standard for malaria diagnosis. Routine microscopic examination is laborious, time-consuming and requires a well-maintained microscope along with experienced microscopist (Mharakurwa et al., 2006). There are numerous malaria rapid diagnostic tests that are commercially available which contains specific antimalaria antibodies to detect malaria antigen (pLDH, HRP-2, p-aldolase) in the blood (Kakkilaya, 2003; Gillet et al., 2009). Immuno-chromatography test (ICT) is a rapid immuno-chromatography assay for the detection of *Plasmodium falciparum* antigen manufactured in a test card form (HRP2). Individuals with fever commonly have elevated levels of protein in their urine, it is therefore not surprising that proteinuria has been reported in patients infected with *P. falciparum*, a disease characterized by cyclic fevers (Boonpucknavig and Sitprija,1979). Ehrich and Horstmann (1985) examine urine samples from seven malaria patients by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and found that *P. falciparum* patients execrated a variety of proteins, it is unknown, however, if any of these proteins are parasite antigens or potentially antimalaria antibodies (Ehrich and Horstmann,1985). In the study of Marina and et al. (1991) the concentration of malaria antigens or antibodies present in freshly collected urine samples should be sufficient for direct use in a diagnostic assay (Marina et al.,1991). Malaria antigens have been detected in the urine, we assessed the efficiency of the QDx rapid

Table 1. Performance of QDx malaria rapid test- blood and urine relative to microscopy malarial parasite.

Samples	Microscope Positive	QDX Blood Positive	QDX Urine Positive
Case (90)	90	83 (92.2%)	16
Control (90)	90	2	0

Table 2. Comparison of performance QDx malaria rapid test-blood with microscopy.

QDX Blood Result	Microscope Result			Sen.	Spec.	PPV	NPV
	Pos	Neg	Total				
Postive	83	2	85	92.2%	97.7%	97.6%	92.6%
Negative	7	88	95				
Total	90	90	180				

(Sen. = Sensitivity, Speci. = Specificity, PPV= Positive Predictive Value, NPV= Negative Predictive Value, Pos. =Positive, Neg. = Negative).

malaria test in the blood and in the urine using microscopy as the gold standard (Genton et al.,1998; Rodriguez-Del Valle et al.,1991).

MATERIALS and METHODS

The study is a cross-sectional study conducted in ELGadaraf hospital, Sudan. A total of 180 suspected patients were enrolled in this study, Blood and urine samples were collected from all cases. Out of these 180 cases, only 90 cases were positive for *P. falciparum* malaria on the blood smear. The other 90 specimens were negative for malaria on blood smear and also using the immuno-chromatography test and the other 90 specimens were used as the control in this study.

Immunochromatographic Test

All the blood samples were processed for QDx rapid malaria test, which is commercially available. It is an immunochromatographic test that detects the presence of specific histidine-rich protein-2 (HRP-2) for the detection of *P. falciparum*. When the blood sample malaria antigens combine with these malaria antibodies, pink-purple colored bands are formed which confirms that test results are positive. The 5 µl of anti-coagulated blood sample or finger pricked blood sample take into sample well, ‘S’. Then six drops of the clearing buffer taken into reagents well, ‘R’. The test results are ready at after 15 min. When only one pink purple band appears in the control window ‘C’ means the blood sample is negative for the malaria infection. When in addition to control band, a pink-purple band appears at the ‘Pf’ region in the test window means the blood sample is positive for the falciparum infection.

Microscopy

All the blood smears were stained with Giemsa stain. Slides were considered positive for malaria when asexual forms and/or gametocytes were found. Slides were negative if no parasites were seen after observing 100 high powered fields. The ‘parasitic density’ calculated as the number of parasites counted on smear was multiplied by the patients’ white blood cell (WBC) count, and the resulting value divided by the total number of WBCS counted during the microscopy examination. Parasites densities were classified into three groups, <500, 501-5000 and >5000 parasites/ microliter. For each slide, parasites were counted against 200WBCS. Manual cell count was done.

Urine

All the urine samples were processed for urine chemical analysis using urine strips to detect proteins in urine. Then all the urine samples (180 cases) were processed by QDx rapid malaria test as in blood sample.

Data Analysis

The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the QDx rapid malaria test for urine and blood were estimated using microscopy as the gold standard by using Open Source Epidemiologic Statistics for Public Health (OpenEpi).

RESULTS AND DISCUSSION

From the 180 blood samples collected, 90 (test samples) were positive for *P. falciparum* by smear examination while the (90) control blood samples were negative smear for malaria. Out of 90 malaria smear-positive cases, 83 (92.2%) blood samples and 16 (17.7%) urine samples were positive for QDx rapid malaria test and proteinuria were detected in 13 cases (Table 1). All 90 controls were negative by QDx rapid malaria test for urine and only 2 blood samples were positive using QDx rapid test while proteinuria was detected in 6 cases in the control samples. When microscopy was compared with QDx Rapid Malaria test, the QDx rapid malaria test processed by blood, it showed a sensitivity of 92.2%, a specificity of 97.7%, PPV of 97.6% and NPV of 92.6% (Table 2). And when microscopy was compared with

Table 3. Comparison of performance QDx malaria rapid test-urine with microscopy

QDX Urine Result	Microscope Result			Sen.	Spec.	PPV	NPV
	Pos	Neg	Total				
Postive	16	0	16	7.7%	100%	100%	54.8%
Negative	74	90	164				
Total	90	90	180				

(Sen. = Sensitivity, Speci. = Specificity, Accu. = Accuracy, PPV= Positive Predictive Value, NPV= Negative Predictive Value, Pos. =Positive, Neg. = Negative).

QDx Rapid Malaria test processed by urine, the sensitivity, specificity, PPV, NPV was 7.7, 100, 100 and 54.8%, respectively (Table 3). The detection of parasites in body fluids rather than the use of blood to determine the presence or absence of parasites could be valuable for communities with blood taboos and reduce compliance problems associated with collection of blood samples. As a result, many researchers had experimented with body fluids like urine, saliva, etc. to detect malaria parasite. Katzin et al (1991), showed that Western blotting technique could detect malaria antigens in urine. Mharakurwa et al. (2006) showed that *P. falciparum* infection could be detected in urine and saliva by PCR technique. Diagnosis is currently been done by microscopy, which requires good training and simple laboratory facilities. On the contrary, the rapid immunochromatographic tests do not require a laboratory, electricity, or any special equipment. They target parasitic antigens histidine-rich protein 2 (HRP2) of *P. falciparum* using either monoclonal or polyclonal antibodies. Histidine-rich protein 2 of *P. falciparum* (PfHRP2) is a water-soluble protein that is produced by the asexual stages and gametocytes of *P. falciparum*, expressed on the red cell membrane surface (Kakkilaya, 2003). QDx rapid malaria test detects malaria antigens of Pf HRP2 in the blood of malaria patients by immunochromatographic principle. Since these malaria antigens are also released in the urine, blood and urine samples of malaria patients on QDx Rapid Malaria test as a test sample was done in this study.

The collection of urine is non-invasive, simple, safe, stress-free, painless, and can be done by individuals with limited training, including patients (Genton et al.,1998; Rodriguez-Del Valle et al.,1991). The QDx rapid malaria test processed by blood gives a sensitivity of 92.2%, specificity of 97.7%. When compared with microscopy as the gold standard, 16 out of the 90 microscopic malaria positive cases were positive by the QDx rapid malaria test using urine samples giving a sensitivity of 7.7%, specificity of 100%, PPV of 100% and NPV of 54.8% (Table 3). As a result of lack of specificity, the QDx rapid test performed on urine cannot be recommended, and the assessment of specificity is difficult in an area highly endemic for malaria because one can never be sure that an individual with a negative thick film is truly parasite free. Genton et al. (1998) showed that when using microscopy and PCR as reference, the ParaSight (R)-F test applied to blood had 84% sensitivity and 77% specificity and when the same test kit was used for urine, it had 81% sensitivity with 26% specificity (Boonpucknavig and Sitprija,1979). Individuals with fever commonly have elevated levels of protein in their urine, but in ELGadaraf state a known malaria-endemic area, few individuals have proteinuria that suggests that *P. falciparum* antigens are not excreted in the urine which indicates more chronic infections rather than acute.

CONCLUSION

The QDx rapid malaria test performed on urine cannot be recommended, the QDx rapid malaria test performed on urine had low sensitivity.

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