Evaluation of immunochromatographic (ICT) assay and microscopy for malaria diagnosis in endemic district Dera Ismail Khan

Zahid Ullah¹, Badshah Noor², Muhammad Faisal Nadeem³, Azam Hayyat³, Aamer Ali Khattak*¹

¹Department of Medical Lab Technology, University of Haripur, KPK, Pakistan
²DHQ Teaching Hospital Dera Ismail Khan, KPK, Pakistan
³Department of Biochemistry and Molecular Biology, University of Gujrat, Gujrat, Pakistan
⁴Department of Microbiology, Hazara University Manshera, Manshera, KPK, Pakistan

Key words: Malaria, Immunochromatographic, Slide microscopy, Plasmodium.


Abstract

This study was designed to compare the specificity and sensitivity of immunochromatographic technique (ICT) for malaria with microscopy of Geimsa stained slide. Dera Ismail Khan (D.I.Khan) is malaria endemic region with problem of accurate, efficient and rapid malaria diagnosis. So to evaluate ICT device efficacy to use as substitute of microscopy in malaria parasite diagnose in remote areas like D.I.Khan. This Study was conducted at a Private Health centre in district D.I.Khan, KPK, Pakistan from March 2014 to October 2014. Four hundreds and seventy patients aged from 03 to 60 years irrespective of gender were included in this study. EDTA whole blood was taken for microscopy and ICT malaria capable of P. falciparum detecting specific histidine rich protein II antigens (Pf.HRP-II) and P. vivax specific lactate dehydrogenase enzyme (Pv.LDH) in patient’s serum. ICT Test procedure were performed and interpreted according to the manufacturer’s instructions. A total 470 patients were enrolled in this study, out of which 194 were found positive for Plasmodium infection, P. falciparum 10 (2.1 %), P. vivax 184 (39 %) and 276 were declared negative by microscopy as well as by ICT method. Data analysis showed that ICT with overall sensitivity of was 100 % (95% confidence interval (CI): 98.10 to 100.00 %), while specificity was 99.28% (95% CI: 97.40 % to 99.89 %). From this study, it is suggested that ICT rapid technique could be used an alternative to microscopy for the diagnosis of malaria in those area where microscopy is not available.

*Corresponding Author: Dr. Aamer Ali Khattak  amir.khattak@hotmail.com
Introduction

Malaria is the major health problem around the world and contributes in high rate of morbidity and mortality. According to the World Health Organization (WHO) approx. 219 million malaria cases have been reported in 2010 and with estimated 0.6 million deaths, 90% of which were from Africa (Department of international development, 2011; Khattak et al., 2013a; WHO, 2013). In Pakistan malaria is serious health problem because malaria peak season (monsoon) overlaps with peak period of agricultural activity. Additionally natural disaster like floods and internally displaced peoples from unrest areas of Pakistan have add more to country’s malaria burden (Mukhtar M, 2009). WHO declared in its 2013 report that annually 0.6 million cases of malaria has been reported and only 2% of Pakistani population lives in malaria free region (WHO, 2013).

Rapid and accurate diagnosis with earlier treatment is solution to reduce morbidity and mortality rate caused by malaria (Smego, Jr. et al. 2000). Presumptive diagnosis and gold standard method of malaria detection i.e. stained slide microscopy are two broadly used methods in many health set ups in Pakistan (Iqbal et al., 2002). But the malaria diagnosis is still a challenge for medical diagnostic lab and an obstacle for efficient malaria control. The search for novel, quicker and easier malaria diagnostic methods has picked up acceleration from the last decade. With innovations of the rapid diagnostic techniques (RDTs) like ICT technique has lead towards new approaches and also calling for evaluation of the existing malarial diagnostic methodologies (Moody, 2002).

From about century microscopic examination of stained thin and thick blood smear is used for malaria parasite examination because it is a cost effective and provides complete information about Plasmodium speciation, level parasitemia and parasitic stages. But this technique has many limitations like time consuming (20-60 minutes), require trained microscopist, microscope with good resolution, quality reagents, time spent on microscopic examination (Thakor, 2000). In the early 1900s ICT malaria test was introduced is based on detection of parasitic antigen(s) in patient’s peripheral blood against antibodies (monoclonal or polyclonal) produced in vitro (Shiff et al. 1993). Malarial antigens at present targeted by RDT are histidine-rich proteins 2 (HRP-2), Plasmodium lactate dehydrogenase (PLDH) and Plasmodium aldolases (Shiff et al., 1993; Rock et al., 1987; Moody, 2002). Although RDTs are quick, easy, do not need any expertise/instrumentations or supervision nevertheless not provides sufficient information about malaria parasite.

Dera Ismail Khan was selected for this study as this region is malaria endemic and factors like migration of internally displaced peoples (IDPs) from unrest regions, socio-economic condition and lack of trained microscopist have added in malaria endemicity. So we supposed that our results might add valuable information. This study was conducted to compare the rapid immunochromatographic technique of malaria diagnosis with slide microscopy.

Materials and methods

Sample collection

The study was conducted at Private Health centre in D. I. Khan district of KPK, Pakistan between from March 2014 and October 2014. D. I. Khan having a warm climate situated on the right bank of Indus River in KPK province of Pakistan. Warm climate, improper drainage system and presence of ponds/lakes provide favorable environment for the propagation of mosquito. Many IDPs from FATA region (malaria endemic region) moved here because of military operation against terrorist. All these circumstances favor malaria endemicity in the region (Khattak et al. 2013b).

Microscopy and RDTs Analysis

Patient selection criteria were, patients with fibril illness with malaria symptoms (vomiting, shivering, ache, headache, pain and abdominal discomfort) were included and patients having any anti-malarial drugs in the last four weeks were excluded from the study. Patient giving informed consent 5 mL of whole blood
sample was collected in EDTA tube. Both thick and thin 10% Giemsa stained blood smears were prepared. All stained slides were examined at 100X under oil immersion objective of microscope by well experienced laboratory technician according to WHO guidelines; at least 200 consecutive fields were viewed before declaring any result as negative.

For RDTs manufacturer’s instructions provided on kit’s leaflet were followed while performed ICT test for *Plasmodium falciparum* and *Plasmodium vivax* (Check at, Haelgen scientific LCC, USA). The test cassette was pre-coated with specific antibodies to histidine rich protein II antigens (Pf HRP-II) of *P. falciparum* and lactate dehydrogenase specific antibodies (Pv.LDH) of *P. vivax*.

**Statistical Analysis**
Collected data was analyzed by SPPS V.19, different variables like number of true negative, true positive, false negative and false positive were measured. To compared ICT and microscopy; sensitivity, specificity, positive predictive value (PPV) and negative predicted values (NPV) for both tests were analyzed by using formula previously described by Tjitra et al. (1999). To check the consistency of the results among the diagnostic tools Kappa values was determine and less than 0.005 $P$ value was considered significant in all comparison.

**Results and discussion**
During the study period of eight months a total of 470 patients were tested simultaneously for microscopic examination and ICT for malaria parasite. Out of these 470 enrolled patients 301 (64%) were males and 169 (36%) females. The age ranges were between 03 to 60 years with median of 31.5 years. Among microscopy positive and ICT 184 (39.1%) cases were positive for *P. vivax*, 10 (2.1%) for *P. falciparum* and 276 (58.7%) cases were found negative for both *P. vivax* and *P. falciparum* and not a single case of *P. malariae* or *P. ovale* was found on microscopy.

<table>
<thead>
<tr>
<th>Result</th>
<th>Microscopy</th>
<th>ICT Malaria</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Plasmodium falciparum</em></td>
<td>10 (2.1%)</td>
<td>10 (2.1%)</td>
</tr>
<tr>
<td><em>Plasmodium vivax</em></td>
<td>184 (39.1%)</td>
<td>184 (39.1%)</td>
</tr>
<tr>
<td>Negative</td>
<td>276 (58.7%)</td>
<td>276 (58.7%)</td>
</tr>
</tbody>
</table>

The overall sensitivity of ICT was 100%, while specificity was 99.28%, with a PPV of 98.98% and NPV of 100%. Two cases of *P. vivax* showed false positive results by ICT, probably due intake of anti-malarial drugs. Between ICT method and stained slide microscopy significant relation has been noted ($p < 0.005$).

The progress in developing new simple, speedy and accurate tests for *Plasmodiasis* detection is highly attractive (Aguilar et al., 2012). RDT commercially introduced during 1994 for the first time, about 200 devices are manufactured by sixty companies are currently being used and efficacies of some are tested by international organization (WHO, 2012). And the field of RDTs has been evolved very fast and constant improvement in its technicalities with raise in their potentials for malaria diagnosis is at rise (Eibach et al., 2013). In our study performance of ICT relative to microscopy was investigated with the samples collected from malaria fibril patients. Slide microscopy is still a gold standard technique in malaria diagnosis with documented detection limit of 50 parasites/μL (0.001% parasitemia) and 98% with speciation differentiation level of all *Plasmodium* species. However, there are certain limitations of this technique i.e. difficult, laborious, lack of adequate quality control, requires skilled staff, need regular microscopy training and inability to cope with patient’s workload in government hospitals (WHO, 2009). In past few decades, efforts have been made to find substitute for the conventional microscopy like RDTs, fluorescence microscopy and PCR which are more sensitive although much expensive and difficult.
for routine busy clinical laboratory use.

The beginning of ICT opened new avenue of more rapid non-microscopic in malaria infection diagnosis (Coleman et al., 2002). In this study we investigated the performance of ICT malaria (Haelgen scientific LCC, USA) devices for the detection of two dominant species P. falciparum/P. vivax of Pakistan with slide microscopy. Overall ICT showed good sensitivity when compared with microscopy. The study demonstrated 100% sensitivity and 99.28% specificity for ICT malaria with PPV of 98.98% and an NPV is 100% our results are in consistent with the results of Huong et al. published in 2002 (Huong et al., 2002). In set ups where health care personnel rely on malarial presumptive diagnosis based on symptoms, ICT can be use for accurate malaria diagnosis and will be helpful in earlier treatment (Iqbal et al., 2002). High values of sensitivity and specificity of the ICT reported in this study emphasis on health care personals confidence. High sensitivity of this study was in line with other study conducted in Sri Lanka (Fernando et al., 2004). The current study showed a higher sensitivity and specificity than reports from Peshawar (Mohammad et al., 2013) Karachi (Harani et al., 2006) and Myanmar (Ashley et al., 2009).

Many studies conducted in clinical setups of different geographical locations also reported comparable level of accuracy of ICT with microscopy (Moody, 2002; Moonasar et al., 2007) but there are few studies in which RDTs showed low sensitivity level (Wongsrichanalai et al., 2007; Marx et al., 2005; Hopkins et al., 2008). Many investigations carried out to evaluate ICT performances showed inconsistent results (Beadle et al., 1994; Durrheim et al., 1998; Iqbal et al., 2002; Jelinek et al., 2000; Moody, 2002; Palmer et al., 1998; Singh et al., 1997). Several factors may affect the diagnostic performance of RDTs such as quality control of the product, temperature, storage, humidity and users’ performance (WHO, 2012). However the sensitivity of all RDTs tests is low when compared with PCR (Iqbal et al., 2002). High NPV shows that ICT is reliable diagnostic test so can be helpful in malaria prevention and interruption in malaria transmission. Higher PPV depicts in this study clearly indicates towards that, ICT can be use for accurate malaria diagnosis to avoid unnecessary antimalarial use.

Stained blood film microscopy is still a gold standard for malaria diagnosis but our study ICT showed parallel results to microscopy in its performance. So it can be use as alternative or supplementary to microscopy for efficient malaria diagnosis in malaria endemic areas where laboratory services are not up to the mark or not standardized with WHO guidelines. ICT can be used in areas where malaria treatment is entirely based on presumptive or clinical diagnosis to reduce antimalarial overuse.

Acknowledgements

The authors thank the study participants for their involvement in the study, and the anonymous reviewers for comments that substantially improved the manuscript. The authors also thank DHQ Teaching Hospital Dera Ismail Khan KPK Pakistan and Hayat Medical Laboratory for their technical assistance in conducting laboratory experiments.

Reference


Mohammad J, Amir S, Rahim F, Khawar N.


