Spatial simulation of malarial infection and it’s diagnosis by RDK

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ABSTRACT
Malaria diagnosis is carried out by microscopic examination of blood films and suspected should preferably be investigated for confirmation of malaria by Microscopy or Rapid Diagnostic Kit (RDK) so as to ensure full therapeutic dose with appropriate drug to all confirmed cases. The aim of the study was to review the spectrum of disease associated with malaria due to P.vivex and P. falciparum in patients presenting to PHC during May, 2008 to Nov., 2009. Among patients admitted with slide-confirmed malaria, 64% of patients had P.falciparum, 24% P.vivex, and 10.5% mixed infections. The proportion of malarial admissions attributable to P.vivex raised to 47% (415/887) in children under one year of age. Severe disease was present in 2,634 (22%) inpatients with malaria, with the risk greater among P.vivex (23% [675/2,937]) infections compared to P.falciparum (20% [1,570/7,817]; odds ratio [OR] = 1.19 [95% confidence interval (CI) 1.08-1.32], p = 0.001), and greatest in patients with mixed infections (31% [389/1,273]); overall p < 0.0001. Severe anemia (hemoglobin < 5 g/dl) was the major complication associated with P.vivex, accounting for 87% (589/675) of severe disease compared to 73% (1,144/1,570) of severe manifestations with P.falciparum (p < 0.001). Pure P.vivex infection was also present in 78 patients with respiratory distress and 42 patients with coma. In total 242 (2.0%) patients with malaria died during admission: 2.2% (167/7,722) with P.falciparum, 1.6% (46/2,916) with P.vivex, and 2.3% (29/1260) with mixed infections (p = 0.126). Between August and November 2007, 1004 patients aged between 1 and 93 years were enrolled in the study. Slide microscopy (the reference standard) diagnosed 213 P.vivex mono-infections, 98 P.falciparum mono-infections and no malaria in 650 cases.

INTRODUCTION
The word malaria comes from 18th century Italian language Mala-meaning “bad” and aria meaning “air”. The term was first used by Dr. Francisco Torti, Italy. Approximately 40% of the global population is at risk of malaria infection. According to WHO the majority of malaria deaths among children in sub-Saharan Africa, killing an African child every 30 second[3]. Malaria is estimated to kill between 1.5 to 2.7 million people each
year with an average of one death every 12 second. The morbidity due to malaria is estimated at around 300-500 million every year[6]. Malaria afflicts approximately 90 countries and territories in the tropical and sub-tropical regions and almost one half of them are in Africa, South of Sahara. In India nine anopheline vectors transmitted malarial species like \textit{P.falciparum}, \textit{P.vivax}, and \textit{P.malariae} in diverse geo-ecological paradigms. In India the states of Orissa Jharkhand, West Bengal, North-Eastern states, Chhattisgarh, Madhya Pradesh contribute bulk of malaria. Most of the malaria attributable mortality is reported from Orissa and other forested areas occupied by ethnic tribes in the country[5]. \textit{P.falciparum} estimates outside Africa especially in South-east Asia are 200% higher than reported by the WHO[8]. \textit{P.vivax} in the world has been calculated at 71-80 million cases of which South East Asia and Western Pacific countries contributed 42 million cases. This study was designed for malaria diagnosis by microscopic examination of blood film and suspected should preferably be investigated for confirmation of malaria by microscopy as well as Rapid Diagnostic kit so as to ensure full therapeutic does with appropriate drug to all confirmed cases. The aim of study was to review the spectrum of disease associated with malaria due to \textit{P.vivax} and \textit{P.falciparum} in patients presenting to PHC during April 2008 to March 2010, and to compare them by microscopic as well as Rapid Diagnostic Techniques.

**MATERIAL AND METHODS**

A finger prick blood sample was collected from each enrolled patient. This sample was used to prepare thick and thin smears from the blood. Thick and thin blood films were prepared and stained with Giemsa following WHO standards. Also blood sample test by using available from SD malarial antigen P.F/Pan, Para check and malaria P.F/P.V antigen.

To prospective study was under taken to study the accuracy of ICT and microscopy for the diagnosis of malarial parasites. Preparation of the smear:

Use universal precautions while preparing the smear for malarial parasites. Use gloves, use only disposable needle/lancets, wash hands, handle and dispose the sharp instruments and other materials contaminated with blood carefully to avoid injury.

1. Whenever possible, use separate slides for thick and thin smears.
2. Thin film (a): Bring a clean spreader slide, held at a 45° angle, toward the drop of blood on the specimen slide.
3. Thin film (b): Wait until the blood spreads along the entire width of the spreader slide.
4. Thin film (c): While holding the spreader slide at the same angle, push it forward rapidly and smoothly.
5. Thick film: Using the corner of a clean slide, spread the drop of blood in a circle the size of a dime (diameter 1-2 cm). Do not make the smear too thick or it will fall off the slide. (You should be able to read newspaper through it.)
6. Wait until the thin and thick films are completely dry before staining. Fix the thin film with methanol (100% or absolute) and let it dry completely before staining. The thick film should not be fixed.
7. If both thin and thick films need to be made on the same slide, fix only the thin film with methanol. The thick film should not be fixed.

**Staining**

Preparation of working buffer (dilute stick buffer 1:10 with distilled water) and preparation of working stain (Giemsa stain 1ml + 9ml working buffer).

1. After spray fixative to blood smear and dry it in air.
2. Pour the working stain on smear and keep for 15-20 minutes.
3. Wash off with Distilled Water or tap water and dry the slide
4. Observe under oil immersion lens and count the cells.

**Examined thick and thin film by microscopy**

**Thick blood film**

Used for detecting malaria a larger volume of blood is examined allowing detection of even low levels of
parasitemia. Also used for determine parasite density and monitoring the response to treatment.

**Thin blood film**

Gives more information about the parasite morphology and, therefore, is used to identify the particular infecting species of plasmodium.

**RESULT AND DISCUSSION**

This prospective study was undertaken to study the accuracy of ICT and Microscopy for the diagnosis of malaria. This study was performed during the period of April-2008 to March-2009 a total of 3064 blood sample collected, out of this 367 sample positive, in which 366 sample is positive by using Microscopy and 365 sample is positive by using RDK. Out of which 248 were caused by *P.vivax*, 119 by *p.falciparum* and one mixed infection. In year of April-09 to March-10 is 8090, out of this 500 sample is positive, In which 498 sample positive by using microscopy and 499 sample is positive by using RDK. Out of which 453 were caused by *P.vivax*, 47 by *P.falciparum*.

**DISCUSSION**

This prospective study was undertaken to study the accuracy of ICT and Microscopy for the diagnosis of malaria. This study was performed during the period of April-2008 to March-2009 a total of 3064 blood sample collected, out of this 367 sample positive, in which 366 sample is positive by using Microscopy and 365 sample is positive by using RDK. Out of which 248 were caused by *P.vivax*, 119 by *p.falciparum* and one mixed infection. In year of April-09 to March-10 is 8090, out of this 500 sample is positive, In which 498 sample positive by using microscopy and 499 sample is positive by using RDK. Out of which 453 were caused by *P.vivax*, 47 by *P.falciparum*.

**CONCLUSION**

We are concluding that immunochromatographic technique is very sensitive and specificity for diagnosis of malaria parasite but cannot diagnosis asexual and sexual form of malarial parasites, while microscopic technique is best to diagnosis of malaria parasite both asexual and sexual form so expert microscopic is best to diagnosis of malaria.

**REFERENCES**