

A Non Invasive Approach for Rapid Malaria Diagnosis from Urine and Saliva of Malaria Patients

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Abstract: The non-invasive, cost-effective malaria tests that minimize the need for blood collection are the need of time. The diagnosis of malaria in biological fluids other than blood would be a valuable approach in case management and epidemiological studies of malaria. Many rapid diagnostic kits are available in the market but till now there is no kit for diagnosing malaria from urine and saliva samples. Keeping these lacunae, this study has given a light to the new non invasive approach for malaria diagnosis for the first time.

Keywords: Malaria diagnosis, non-invasive, urine, saliva.

I. INTRODUCTION

Malaria remains a major public-health problem in India. Early diagnosis and appropriate treatment are essential for addressing the global burden of malaria. Presently all malaria diagnostic methods microscopy and rapid diagnostic tests (RDTs) are invasive as they depends on blood samples for diagnosis (Fig.1) WHO[1]. These invasive methods of repeated drawing blood sample by inevitable use of needle especially from young children who experience the highest malarial burden is difficult and leads to extra load in field study Moody [2]. Also these techniques need rigorous training and biological safety precautions to ensure proper containerization, disposal of used needles and proper preservation of blood specimens from the field to well-equipped laboratory which influence the performance of the tests. Mishandling of needles otherwise leads to the risk of accidental infection such as diseases like tuberculosis, AIDS etc. Diagnostic strategies, thus, need to be simple, practical, and applicable, especially in malaria-endemic and resource-poor regions of India. It has recently been found that it is possible to detect malaria parasite antigens/DNA in samples of urine and saliva from malaria patients. Till now, no work has been carried out or reported for development of antibody based diagnostic kit to detect these antigens from urine and saliva of malaria patient. Thus, there is an urgent need to develop a non-invasive rapid method of malaria diagnosis using body fluids other than blood which may facilitate access to accurate, non invasive malaria diagnosis in areas where this was not previously possible.

II. ESTIMATED REQUIREMENT

According to the recent World Malaria report [3], over 70% of India's population, about 100.41 crores face the risk of malaria infection (Fig 2). Around 31 crores, however, face the "highest risk" of getting infected by the vector borne disease. India has over 10 crore suspected malaria cases, but only 15.9 lakhs could be confirmed in 2010. Of the confirmed cases, 8.3 lakhs were infected by plasmodium falciparum, while 7.6 lakhs were infected with Plasmodium Vivax (www. malaria\India to raise malaria toll figure 40-fold - Times Of India.mht) [4]. On interaction with users and market surveys we estimated 20 lakhs cases of malaria in endemic and non endemic zones of India.

III. INTERNATIONAL STATUS FOR THIS APPROACH

Malaria breeds poverty and underdevelopment in vast regions of the world, including the Americas, thus contributing to issues of global concern such as illegal migration and security. Nearly 142 million people or 16% of the population of the Americas is among those who are at risk for malaria and 40 million of them are at moderate to high risk. Rolling back malaria is possible. In the Americas, miners, loggers, banana and sugarcane plantation workers, indigenous groups, populations in areas of armed and/or social conflict, and people along areas of common epidemiologic interest / border areas are also susceptible to the disease. Large numbers of Rapid diagnostic kits (RDTs) have been developed for early diagnosis of malaria by different researchers [5],[2][6]. However, all the current methods are invasive as they require blood for diagnosis. It has recently been found that it is possible to detect malaria parasite antigens/DNA in samples of urine and saliva from malaria patients [7-8]. But the existing lacunae is that till now, no work has been carried out or reported for development of antibody based diagnostic kit to detect these antigens from urine and saliva of malaria patient.

IV. NATIONAL STATUS FOR THIS APPROACH

In India currently all malaria diagnostic methods (microscopy and RDTs) are invasive as they depend on

blood samples for diagnosis. These detection methods are time-consuming, usually of 2-3 days culture and phenotyping. There are few kits imported from foreign countries like BinaxNow malaria test kit, ACCU-TELL MALARIA P.F/P.V WHOLE BLOOD TEST (CE) is available, but they are not cost effective in India and also none of them are invasive i.e diagnose by using urine and saliva of the patients. Hence there is an immense significance in developing such non invasive, rapid, highly sensitive, cost effective malaria detection kit in India that serves the needs of scientist of the bioresearch, hospitals and health care markets.

V. PRINCIPAL OF OPERATION

The technology utilizes the principle of immunochromatography. As the test sample (urine/saliva) flows through the membrane assembly of the device after addition of the clearing buffer, the colored monoclonal antibody complexes the antigen in the lysed sample. This complex moves further on the nitrocellulose membrane to the test region where it is immobilised by the anti vivax specific monoclonal antibody and/ or the anti falciparum specific antibody coated on the membrane leading to formation of pink-purple colored band/s which confirms a positive test result. A band will appear under Pf at the test region in falciparum malaria positive samples, while a band will appear under Pv in vivax malaria positive samples. Appearance of band under Pf as well as Pv in the test region suggests a mixed infection. Absence of colored band/s in the test region indicates a negative test result. The unreacted conjugate and unbound complex if any move further on the membrane and are subsequently immobilised by anti rabbit antibodies coated on the membrane at the control region, forming a pink-purple band. This control band serves to validate the test performance (Fig 3).

VI. DISCUSSION

In accord with current knowledge of malarial parasite life cycle, detection or screening for malaria infection presupposes the drawing of blood by finger prick or vevpuncture. The need to draw blood causes difficulties in certain communities with blood taboos and poses workload and cost in field situations [7]. For these reasons, many researchers had experimented with the body fluids like urine, saliva etc. for detection of malaria. Mharakurwa et al described the use of urine and saliva samples for PCR detection of Plasmodium falciparum infection and illustrate potential application in genotyping malaria parasites. Alejandro *et al.* [9] showed that by Western blotting technique the antigens were detected in the urine of patients infected with Plasmodium falciparum and plasmodium vivax. Diagnosis is currently done by microscopy, which requires good training and simple laboratory facilities. Although the peripheral blood smear examination that provides the most comprehensive information on a single test format has been the 'gold

standard' for the diagnosis of malaria, the immunochromatographic tests for the detection of malaria antigens, developed in the past decade, have opened a new and exciting avenue in malaria diagnosis. These immunochromatographic tests do not require a laboratory, electricity, or any special equipment. Currently, immunochromatographic tests can target the histidine-rich protein2 of *P. falciparum*, a pan-malarial Plasmodium aldolase, and the parasite specific lactate dehydrogenase.

Histidine – rich protein2 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, and shown to remain in the blood for at least 28 days after the initiation of antimalarial therapy. Parasite lactate dehydrogenase (pLDH) is a soluble glycolytic enzyme produced by the asexual and sexual stages of the live parasites and it is present in and released from the parasite infected erythrocytes. It has been found in all 4 human malaria species, and different isomers of pLDH for each of the 4 species exist. With pLDH as the target, a quantitative immunocapture assay, a qualitative immunochromatographic dipstick assay using monoclonal antibodies, an immunodot assay, and a dipstick assay using polyclonal antibodies have been developed Kakkilaya [10]. The collection of urine is non-invasive, simple, safe, stress free, painless, and can be done by individuals with limited training, including patients. No special equipment is needed for collection and it allows for multiple or serial collections outside of the hospital.

VII. CONCLUSION

Detecting parasite antigens in urine to determine presence or absence of parasites could be valuable for communities with blood taboos and reduce compliance problems associated with collection of blood. Furthermore, it will provide a cost-effective approach for the screening of large populations in epidemiological surveys while being affordable, rapid, non-invasive, and safe for patients and technicians in resource-poor environments.

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Figure:1. Existing non-invasive methods for diagnosis of malaria

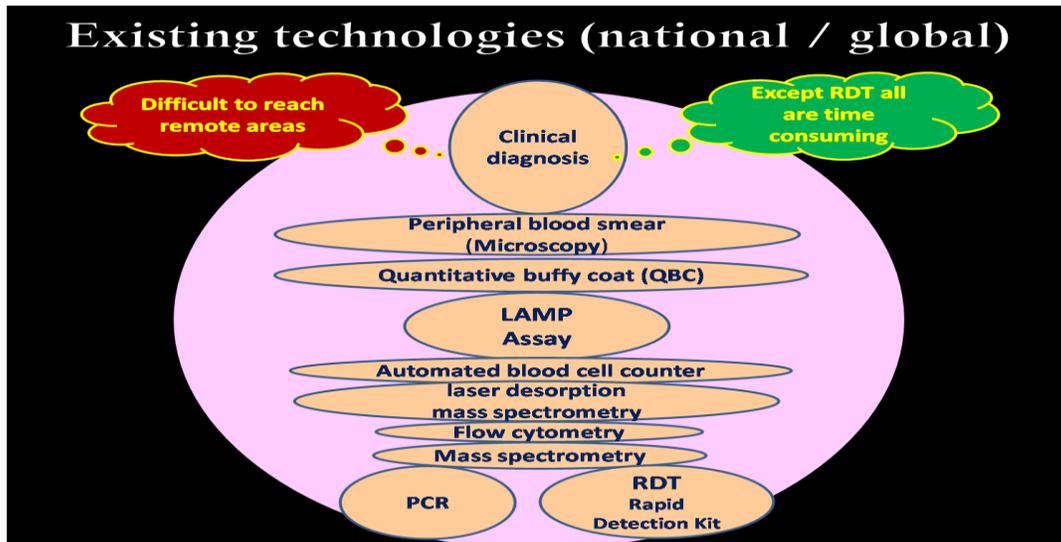


Figure:2. Present scenario of malaria in India

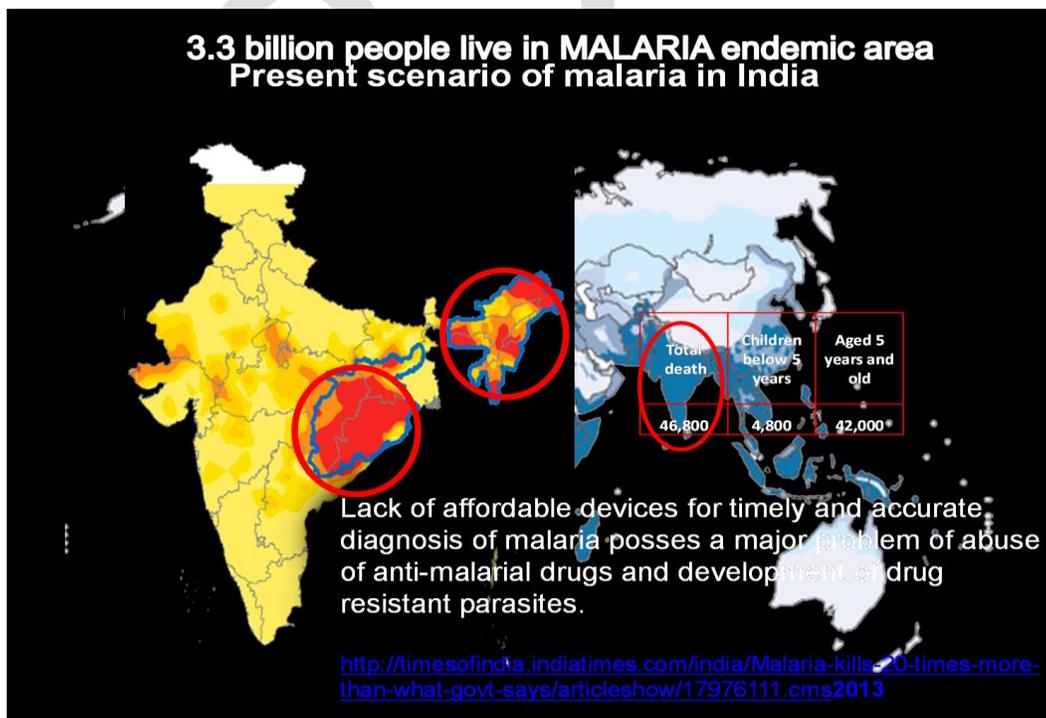


Figure:3. Design and working Principle of the Device

