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COMPARISON OF THREE TECHNIQUES FOR THE DIAGNOSIS OF MALARIA IN RESOURCE-LIMITED SETTINGS: A MINI-REVIEW

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ABSTRACT: Objective of this article is to review three techniques, routinely used rapid diagnostic tests (lateral flow immune chromatography) versus nucleic acid amplification test (NAT) versus Paper-based microfluidics for DNA diagnostics of Malaria, as diagnostic tests in detecting malarial infection among febrile illnesses, suspected of malaria. The major constraint for implementing NAT as a routine screening technique in India appears to be its high cost per test. There is a need to evaluate the technique and its cost-effectiveness as compared to RDT in the Indian setting as there is a scarcity of literature in this area.

INTRODUCTION: Malaria is a protozoan disease transmitted by the bite of infected *Anopheles* mosquitoes. It is transmitted in 106 countries containing 3 billion people and causes approximately 2000 deaths each day; mortality rates are decreasing as a result of highly effective control programs in several countries. Malaria has been eliminated from the United States, Canada, Europe, and Russia; in the late twentieth and early twenty-first centuries; however, its prevalence rose in tropical areas of Africa, Asia and Latin America, where it contributes as one of the world's greatest public health problems.

Prevalence of Malaria: As per WHO report 2017, only eight percent of malaria cases were diagnosed in 2016 in India, which constituted six percent of the 216 million new cases globally.

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The report also suggested that India was the third on the list of 15 countries which accounted for 80 percent of all malaria cases in the world in 2016. According to the report, only 8 percent of total malaria cases were detected in India due to poor surveillance mechanisms [1].

According to World Health Organisation, in South East Asia Region, 70% of malaria cases were from India. Global estimate shows developing countries report a maximum number of cases of malaria, with the majority of infected people living in urban India. When we consider the prevalence of malaria in Karnataka, a study reports that Dakshina Kannada, a southern coastal district of Karnataka State, contributes almost 50% of the cases to the state malaria profile [2].

As screening techniques used for malarial parasite detection have their own drawbacks, it is justifiable to compare their sensitivity, specificity, and cost-effectiveness.

Diagnosis of Malaria: The Giemsa-stained blood slide using thin and thick smears for malaria parasites has been the gold standard method for

nearly a century [3]. No alternative method still could be established to replace this universally accepted gold standard method. This technique to confirm the clinical suspicion of malaria is labor-intensive [4] and sometimes unreliable due to lack of skilled microscopists, limited supplies, inadequate maintenance of microscopes and reagents and inadequate or absence of quality-control systems [5].

In recent time, lateral flow immunochromatographic based rapid diagnostic tests (RDT) have been developed for the diagnosis of suspected malaria patients and are widely used in remote areas across the world [6]. Rapid diagnostic test (RDT) kits offer great potential for the immediate diagnosis of malaria infections. Rapid diagnosis ensures prompt treatment, especially in rural settings. RDTs are lateral flow immunochromatographic tests that detect Plasmodium parasite antigens in blood [7]. Three antigens are detected by current RDTs: histidine-rich protein 2 (HRP2), lactate dehydrogenase (LDH) and aldolase [8].

However, the performance of such assays has been limited by their generation of false-positive results, which occur when nonspecific biomolecules present in the blood, such as the rheumatoid factor, for example, react with the test antigens. Technical issues linked to capillary flow and to reagent stability in challenging environmental conditions, including high humidity and temperature [9], have also affected the reliability of RDT immunodiagnostics. Test sensitivities are often only 70 to 75% in the field, despite being much higher in well-controlled laboratory conditions.

Nucleic Acid Testing (NAT) and Malaria: Nucleic acid amplification-based tests (NAATs) provide a promising approach for DNA-based malaria diagnostics. These tests both amplify and detect the genomic material of the parasite directly from a patient sample, providing a sensitive, species-specific test that will also identify whether an infection is current rather than historical. In addition, the signal used for detection does not depend on the patients' immune responses, and the technique can be both quantitative and more accurate than Immuno-RDTs [10].

Several nucleic acid testing (NAT) approaches for the diagnosis of human malaria infection have been developed in the past two decades [11]. Studies suggest that NAT can detect and quantify parasites more sensitively and precisely than by microscopy or rapid diagnostic tests (RDTs). NAT is valuable for controlled human malaria infection studies of investigational drug and vaccine candidates, for drug efficacy studies as well as for epidemiological surveillance [12]. Parasite load can be quantified by NAT assay 2–6 days earlier than microscopy [13].

In many reference laboratories, PCR-based amplification assays remain the gold-standard NAAT [10], although the requirement for trained staff and external power has limited their application in areas with reduced resources.

In recent times, the real-time PCR method has been established for the quantitative detection of malaria parasites [14]. Real-time PCR is reliable and yields high sensitivity and specificity when compared with microscopy or nested PCR [15].

In a Chinese study, Yan *et al.,* compared the sensitivities of RDTs and microscopy with that of nucleic acid testing by nested PCR. The study concluded that Compared to PCR, both microscopy and RDTs had lower sensitivities, especially for *P. vivax* diagnosis [16]. A study by Alam *et al.,* in Bangladesh compared the sensitivities of rapid diagnostic tests and nucleic acid amplification tests, and the study concluded that SYBR Green-based real-time PCR assay could be used as an alternative gold standard method in a reference setting. Commercially-available RDTs used in the study are quite sensitive and specific in detecting *P. falciparum*, although their sensitivity in detecting *P. vivax* was not satisfactory compared to the real-time PCR assay [17]. Perandin *et al.,* reported better sensitivity with RT PCR in detecting malarial parasites as compared to RDT [18].

The major constraint for implementing NAT as a routine screening technique in India appears to be its high cost per test and the time duration required.

Use of a device, Paper-based microfluidics for DNA diagnostics that uses origami to enable multiplexed, sensitive assays that may be superior to PCR-based laboratory assays and provide high-quality, fast precision diagnostics for malaria. The paper-based microfluidic technology combines vertical flow sample-processing steps, including paper folding for whole-blood sample preparation,

with an isothermal amplification and lateral flow detection, incorporating a simple visualization system. Aim of the study would be to review the diagnostic sensitivity and specificity of paper-based microfluidics for DNA diagnostics of malaria. We hypothesize that advanced, low-cost DNA-based sensors can be implemented in underserved communities at the point of need cost-effectively.

Cost-effectiveness Analysis: Cost-effectiveness analysis is an important tool to assist clinicians, scientists, and policymakers in determining the efficiency of healthcare interventions, guiding societal decision-making on the financing of healthcare services, and establishing research priorities. Diverse approaches to synthesize evidence have been considered in biomedical research, including economic evaluations of healthcare interventions [10, 19]. At the same time, decision-making in health care requires an understanding of the state of economic evaluation at a national level, where the completeness of the reporting is generally less well understood but where specific priorities are often set. Cost-effectiveness analysis (CEA) compares two diagnostic tests, where the costs are identified in monetary terms and the outcomes in non-monetary terms.

Measurement of cost-effectiveness could be made in two different ways:

1. ACER – Average Cost-Effectiveness Ratio
2. ICER – Incremental Cost-Effectiveness Ratio

It helps a decision-maker to compare one treatment/diagnostic test to another, thereby quantifying the opportunity cost of decisions.

Future Research Perspective: The study that can demonstrate that paper-based microfluidic devices can deliver precision diagnostics for malaria in low-resource, underserved settings with a sensitivity that is higher than that of the current malaria diagnostic tests used in the field and with performance that is similar to that of a laboratory-based real-time PCR test is the need of the hour. These diagnostic devices could have a meaningful, positive impact on the provision of mass screening and treatment in campaigns to eliminate the infectious disease. These campaigns have had limited success to date in combating malaria transmission, which has been linked to the inability of current field-based diagnostic tools to detect low-level infections. Thus, the availability of easy-to-use, highly sensitive NAATs, such as those provided by this device, could potentially detect these missed cases and reduce the opportunity for transmission. This would have a significant impact on public health in areas where malaria is highly prevalent.

Impact of the Study: The study may demonstrate that paper-based microfluidic devices can deliver precision diagnostics for malaria in low-resource, underserved settings with a sensitivity that is higher than that of the current malaria diagnostic tests used in the field and with performance that is similar to that of a laboratory-based real-time PCR test. These diagnostic devices may have a meaningful, positive impact on the provision of mass screening and treatment in campaigns to eliminate the infectious disease. These campaigns have had limited success to date in combating malaria transmission, which has been linked to the inability of current field-based diagnostic tools to detect low-level infections. Thus, the availability of easy-to-use, highly sensitive NAATs, such as those provided by this device, could potentially detect these missed cases and reduce the opportunity for transmission. This would have a significant impact on public health in areas where malaria is highly prevalent. It could also inform current thinking within governments and non-governmental organizations concerning improvements in the effectiveness and cost-effectiveness of prophylactic approaches to control diseases (where new precise diagnostic tools are required to rapidly and accurately target where treatment is needed).

Economic assessments of diagnostic tests are inherently difficult than assessments of therapeutic interventions because of uncertainty about the relation between diagnosis and end result or outcomes of care. Towards the end, this study would evaluate the economic feasibility of the introduction of paper microfluidic technique for DNA diagnostics as a diagnostic test for malaria. The economic evaluation of the cost-effectiveness of NAT using the Yield of the NAT test vis a vis the conventional RDT test would have profound implications with respect to policymaking and utility of the test for diagnosing individuals with

malaria. If the malarial infection detection rate by paper microfluidic technique for DNA diagnostics were to be proven to be beneficial, it would pave new roads for early diagnosis of the disease by providing scientific evidence for possibly implementing this test as a useful diagnostic test. This study would help in planning out further strategies for the effective management and treatment of individuals detected by the test. It would also dive into newer research areas to establish the subsequent decrease in the morbidity and mortality associated with malaria given appropriate facilities for early treatment after detection would be mandated at the policy level.

CONCLUSION: The Implications of this study from the patient's perspective would mean early diagnosis which forms the tenet of control of the disease by increasing the yield. Early diagnosis at community level would translate into application of efficient prevention mechanisms to spread the infection. The cost effectiveness analysis would provide scientific basis for adoption of the best test for the diagnosis, given the economic feasibility of the study. Early diagnosis will aid the clinician in providing timely treatment by reducing the morbidity and mortality due to malarial infection.

National Relevance: The Implications of this study from the patient's perspective would mean early diagnosis, which forms the tenet of control of the disease by increasing the yield. Early diagnosis at community level would translate into application of efficient prevention mechanisms to spread the infection. The cost-effectiveness analysis would provide a scientific basis for the adoption of the best test for the diagnosis, given the economic feasibility of the study. Early diagnosis will aid the clinician in providing timely treatment by reducing morbidity and mortality due to malarial infection.

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