COMPARATIVE EVALUATION OF MALARIA ANTIGEN TEST AND PERIPHERAL BLOOD SMEARS IN DIAGNOSIS OF MALARIA

Dr. Ravi Ranjan Singh[1*], Mrs. Kumari Pallavi[2], Dr. Sanjeev Ranjan[3]

Abstract

Background: Malaria is a prevalent, fatal illness in endemic places that poses a diagnostic challenge to laboratories in the majority of endemic countries. A prompt and precise diagnosis is a need for successful therapy, particularly in cases of potentially deadly P.falciparum infection. Though for many years regarded the gold standard for diagnosing malaria, microscopic blood analysis is highly labor-intensive and requires sufficient technological know-how and personnel. Due to this, rapid detection tests for malaria (RDT) based on the detection of malarial antigen in whole blood have been developed.

Aim: Comparison of the peripheral blood smear test from pyrexia of unknown cause with the malaria antigen card test

Material and methods: This was a prospective study conducted from October 2022 to March 2023 . The study involved people who visited the outpatient department with a fever, chills, and rigors. 350 patient samples were collected during the period. Both peripheral smear and Rapid diagnostic tests performed on the same blood sample. Blood smear were made and stained with Leishman Stain, which were then meticulously checked for the presence of the malaria parasite under an oil immersion. According to the manufacturer's instructions, every sample was put through the Advantage MAL Card by J. Mitra test for malaria antigen.

Results: 75 of the 350 peripheral smears examined revealed the presence of the malaria parasite. One instance had Plasmodium Falciparum (Pf) and one smear revealed a mixed infection with both Plasmodium Vivax and Plasmodium Falciparum. Plasmodium Vivax (Pv) was found in 73 cases. Rapid Diagnostic testing revealed 80 positive cases, of which 76 were caused by Plasmodium Vivax, 02 by Plasmodium Falciparum, and 02 by combination infections of the two

Conclusion: Our analysis demonstrates that the malaria antigen card test is an easy, trustworthy, and quick method for determining the parasite's species as well as its diagnosis. The test may be a viable addition to microscopy at tertiary care facilities as well as a prospective replacement for it in our nation's distant and rural areas.

[1*]Assistant Professor, Narayan Medical College and Hospital, GNSU, Sasaram
[2]Tutor, NPIAS, GNSU, Sasaram
[3]Senior Resident, Narayan Medical College and Hospital, GNSU, Sasaram

***Corresponding Author:** Dr. Ravi Ranjan Singh
*****Assistant Professor, Narayan Medical College and Hospital, Sasaram. email: ravisingh_1@rediffmail.com

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Introduction

One of the major diseases spread by vectors in India is malaria. If not identified and treated promptly, it could be fatal. 89% of people in India live in malaria-prone areas, with 22% living in high transmission areas (> 1 case per 1000 people) and 67% in low transmission areas (1 case per 1000 people). According to the National Vector Borne Disease Control Program and WHO there are presently 0.7–1.6 million malaria cases that have been confirmed, resulting in 400–1,000 annual deaths[1]. Five Plasmodium species with varying geographic distributions cause malaria; but Plasmodium vivax and Plasmodium falciparum are more prevalent in India[2].Tests with improved sensitivity and specificity are required to confirm malaria. Smear microscopy is still the best approach for diagnosing malaria compared to other recently developed techniques. Good findings are produced by a high-quality microscope and skill in locating microorganisms. RDTs are increasingly employed for malaria diagnosis, particularly in areas without access to microscopic equipment. Approximately 200 distinct RDT kits with a variety of specificity and sensitivity are marketed commercially[3]. However, any diagnostic test must have >95% sensitivity in order to be helpful as a screening test. The purpose of this study was to compare the effectiveness of the Rapid Diagnostic test and the peripheral smear examination for the diagnosis of malaria.

Aim

Comparison of the peripheral blood smear test from pyrexia of unknown cause with the malaria antigen card test

Methods and Materials

This was a prospective study conducted in the department of Pathology at Narayan Medical College and Hospital, Sasaram. Duration of the study was from October 2022 to March 2023. The comparison study involved people who visited the outpatient department with a fever, chills, and rigors. 350 patient samples were collected from October 2022 to March 2023. Both peripheral smear and Rapid diagnostic tests performed on the same blood sample. Thick and thin smears from EDTA Blood were made and stained with Leishman Stain, which were then meticulously checked for the presence of the malaria parasite under an oil immersion. According to the manufacturer's instructions, every sample was put through the Advantage MAL Card BY J. Mitra test for malaria antigen. An immunoassay based on the "Sandwich principle" is the Advantage MAL Card. The compound contains monoclonal anti-pan specific pLDH (plasmodium lactate dehydrogenase) antibody coupled to colloidal gold. Monoclonal anti-Pf pLDH antibody and monoclonal anti-Pan specific pLDH antibody immobilized on nitrocellulose strips are used in the test. Anti-coagulated blood(EDTA) was used for the examination. According to the manufacturer's instructions, the procedure was followed. With the use of the disposable loop included with the kit, approximately 5 µl of blood were added to the sample well. The second well received 4 drops of the kit's included assay diluents. Result interpretation took place after 15–20 minutes. Results were regarded as negative when just the control band emerged and two of the test bands were negative, and as mixed infection when both the control band and the test bands were visible. Plasmodium Vivax infection was assumed when the PV band appeared alongside the control band. Upon the appearance of the Pf band and control band, Plasmodium Falciparum was identified[4].

Interpretation of Advantage MAL Card test result was done as below:

only control band	Negative
one control band and one test band	Positive: either P.vivax or P. falciparum ● Plasmodium Vivax: when PV band appeared along with control band. ● Plasmodium Falciparum: when Pf band and control band appeared)
one control band and two test bands	Positive: P.vivax and P. falciparum

Inclusion Criteria:

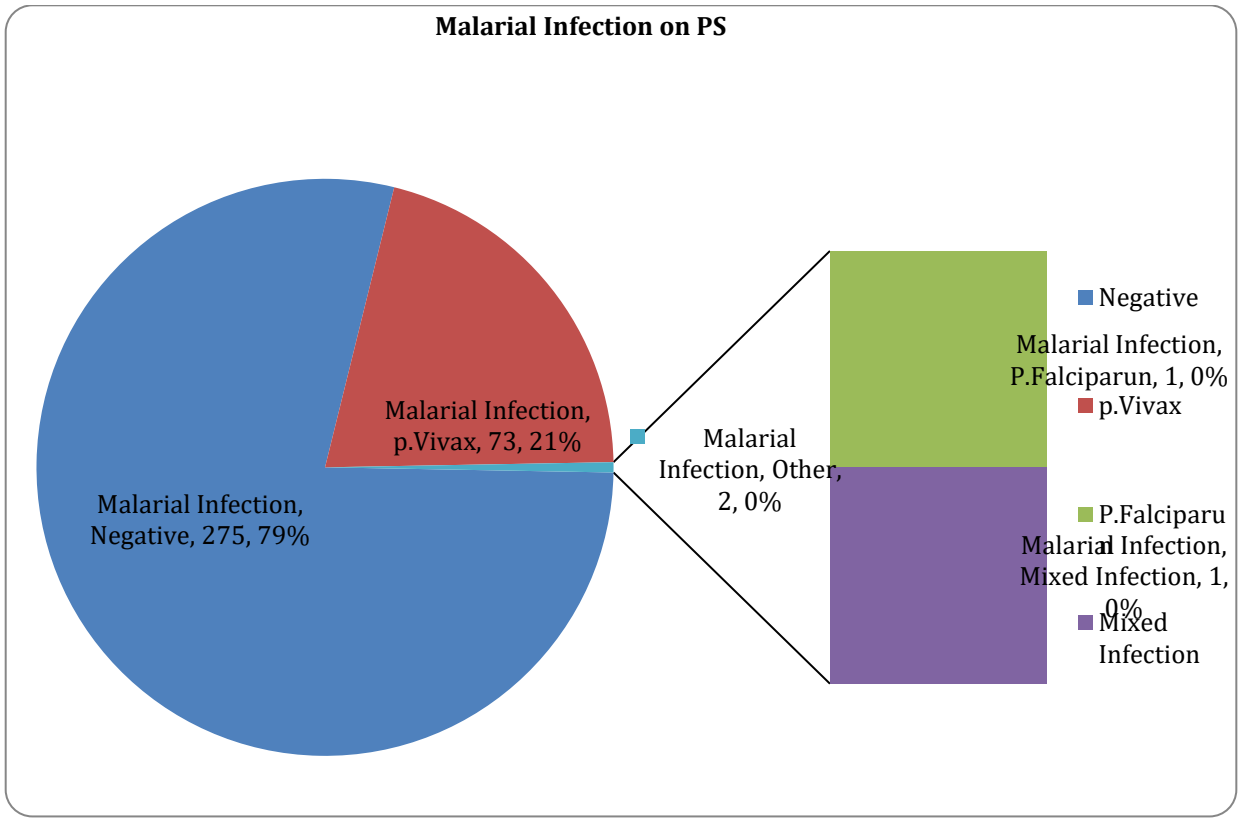
Clinically suspected cases of malaria

Results:

In the current investigation, 350 samples underwent a traditional peripheral smear examination and a Rapid Diagnostic Test to determine whether a malaria parasite was present. 75 of the 350 peripheral smears examined revealed the presence of the malarial parasite. One instance had Plasmodium Falciparum (Pf) and one smear revealed a mixed infection with both Plasmodium Vivax and Plasmodium Falciparum. Plasmodium Vivax (Pv) was found in 73 cases. Rapid Diagnostic testing revealed 80 positive cases, of which 76 were caused by Plasmodium Vivax, 02

by Plasmodium Falciparum, and 02 by combination infections of the two.

Result	Periphearl Blood Smear	Advantage MAL Card test (Rapid Diagnostic Test)
Positive	75	80
P.Vivax	73	76
P.Falciparum	01	02
Mixed infection	01	02
Negative	275	270
Total	350	350



Discussion

In India, malaria is a serious public health issue that causes significant morbidity, death, and monetary losses. Malaria is a parasitic infection with significant worldwide implications. Early detection and timely treatment of cases are the goals of the monitoring efforts against malaria in order to lower attributable morbidity and death. To lower malaria-related mortality and morbidity, accurate malaria diagnosis and prompt treatment are crucial. several methods for diagnosing malaria are peripheral smear, quantitative Buffy coat, antigen-based Rapid diagnostic kits, and polymerase chain reaction (PCR) . Rapid detection tests are an essential weapon in the battle against malaria because they may be used with little to no training and in low-resource settings, unlike PCR-based and microscopic diagnostics, which both need professional staff with extensive training. Microscopy and PCR are less useful in locations with low resources due to outdated or nonexistent equipment and unreliable power, in contrast to rapid detection tests, which don't need an electrical supply, specialized training, or large, expensive lab

equipment[5]. The sensitivity of microscopic testing is less than 75%, according to a 2011 WHO study. In many regions of India, it is standard practice to treat feverish patients with antimalarial medications even after a negative microscopic test, which has led to resistance to the widely used medicine chloroquine. Now, if empirical therapy is used, there is a risk of the development of drug resistance to artemisinin therapy. Additionally, because artemesinin is more expensive than chloroquine, empirical therapy may not be cost-effective.

The current preliminary proposal from WHO is to employ parasite-based diagnosis in all suspected cases of malaria, maybe with the exception of children in high-prevalence regions and in some specific circumstances. Evidently, a quick and precise laboratory diagnosis or evidence of the malaria parasite is required for this guideline to be followed[1].

In endemic locations where there is a dearth of skilled personnel, particularly in rural parts of India, rapid diagnostic tests (RDTs) for malaria might be considered for the majority of patients. But there isn't much data, particularly from malaria-endemic regions, to help decision-makers understand the sensitivity and specificity of these RDTs. RDTs are commercially accessible in kit form with all required reagents, and due to the simplicity of the processes, they may be carried out without specialized training or equipment and the findings are easy to interpret. In 12–15 minutes, the results are read[6].

In a recent external quality control session, 72.7% of 183 Belgian laboratories providing malaria diagnosis claimed using RDTs as a tool for diagnosis, and its usage is advised if done in conjunction with microscopy. In addition, Maltha et al. demonstrated that P. falciparum, Plasmodium vivax, and Plasmodium malariae demonstrated 94.6%, 92.9%, and 94.7% degree of sensitivity using RDTs in malaria parasite concentrations of less than 1,000/L, respectively, but that they demonstrated percentages less than an average of 58% sensitivity in malaria concentrations of less than 100/L. Naturally, it should be anticipated that at a concentration of 0.001% (50/L), where microscopy should also be negative, their sensitivity will decline to practically nil[7].

Advantage MAL Card is an immunoassay based on the "Sandwich principle". Colloidal gold is combined with a monoclonal anti-pan specific pLDH (plasmodium lactate dehydrogenase) antibody in the conjugation. The compound includes monoclonal anti-pan specific pLDH (plasmodium lactate dehydrogenase) antibody coupled to colloidal gold. Monoclonal anti-Pf pLDH antibody and monoclonal anti-Pan specific pLDH antibody immobilized on nitrocellulose strips are used in the test. The apparatus receives the test sample. Red blood cells are lysed when test buffer is added. The P.f specific pLDH/Pan specific pLDH in the lysed sample is complexed by the colloidal gold conjugate if the sample includes P.falciparum or P.vivax/ P.malariae /P.ovale. By means of capillary action, this complex moves through the nitrocellulose strip. A purplish pink band that verifies a reactive test result is formed when the complex is trapped when it encounters the line of the appropriate immobilized antibody. A negative test result is shown by the absence of a colored band in the test location. An extra line of anti-mouse antibody has been fixed on the strip as a control. As per the data obtained from Advantage Mal card, For P. falciparum (pLDH), the test may identify parasitemia levels of >100 parasites per l of blood and >200 parasites per l of blood for P. vivax (pLDH). The Advantage MAL CARD was compared with microscopic inspection and internally assessed using clinical whole blood samples for malaria positivity and negativity.

RDTs are more sensitive and specific for P Falciparum and mixed infection detection as compared to peripheral smear. This is significant because P. Vivax requires treatment with primaquine to avoid malaria relapses, whereas Falciparum produces severe illness and has a high death rate necessitating immediate intervention. RDTs have the advantages of being straightforward, simple to use, requiring no special equipment or electricity, and being simple to understand. However, because parasite density might remain positive for 7–14 days after therapy, it cannot be measured and cannot be used to gauge treatment response [10]. Additionally, choosing which RDT kit to use is a constant source of uncertainty due to the more than 60 brands that are offered in India. Pf/Pan-specific RDTs are unable to distinguish between mixed infections.

Although the peripheral smear is the least expensive of the two, it is more difficult to execute, less sensitive, and requires a microscope, electricity, and a trained technician to interpret. Moreover the smears quality will determine the results. However, the benefits of peripheral smears are that they are more affordable than RDT, allow for the assessment of parasite density, and may also be used as a quality control tool to evaluate the effectiveness of RDTs[8].

Conclusion

The gold standard for diagnosing malaria is said to be peripheral smears. RDTs have the potential to be more sensitive and focused than peripheral smears. Older Pf/Pv specific antigen cards cannot differentiate between mixed and PF infections. To evaluate the effectiveness and cost-effectiveness of various RDTs, however, more research is needed.

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