DIAGNOSIS OF MALARIA BY RAPID DIAGNOSTIC TEST (MALASCAN) WITH SUSPECTED MALARIA AND ITS COMPARISON WITH ROUTINE MICROSCOPY-A HOSPITAL-BASED STUDY.

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Abstract

Introduction: *Sundargarh, a severely impacted tribal region in the state, faces meso- to hyper-endemic malaria, primarily caused by p. falciparum, accounting for 80-90% of cases. The Malascan test, detecting HRP-2 and pan-specific aldolase, offers a quick and simple way for untrained personnel to identify malaria within 10-15 minutes. Early identification, especially of p. falciparum, can significantly prevent further malaria-related complications. This research aimed to compare the accuracy of Malascan (ICT) against routine microscopy (considered the gold standard) in malaria diagnosis.*

Materials and Methods: *This comparative study was conducted in Ispat general hospital , Rourkela, Odisha in children under 15 years of age. ICT was done in all cases in pediatric ward. Thick and thin smears were prepared under standardized protocols and microscopy was done in malaria research laboratory in IGH.*

Results: *Our study has showed that "malascan" had excellent sensitivity (95.16%) and specificity (98.24%) for detecting malaria. Malascan test had positive predictive value of 98.33%, negative predictive value of 94.91% with p value <0.0001.*

Conclusion:*The study findings underscored the Malascan test's effectiveness in detecting malaria among children under 15, surpassing routine microscopy. Its strong accuracy in both identifying positive and negative cases suggests its potential as a reliable tool for prompt and precise malaria diagnosis, offering a promising alternative in healthcare settings.*

Keywords: *Under 15,* MALASCAN, Rapid Diagnostic Test, Malaria

Introduction:

Malaria continues to pose a major public health threat worldwide and in India, particularly due to plasmodium falciparum which is prone to complications and is responsible for most of the mortalities due to malaria. In 2011, total malaria cases were 1.31 million out of which 463 deaths were reported [1]. Most of the total reported deaths were children less than 5 years of age and most of the deaths were attributed to plasmodium falciparum. Falciparum has gradually increased from 38.8% in 1995 to nearly 50.3% in 2011, which may indicate increasing resistance to chloroquine. Malaria stands as a critical health concern in Odisha, notably contributing to a significant portion of both cases and fatalities, despite the state representing a mere 4% of India's total population. The Sundargarh district, nestled within the tribal regions, grapples severely with malaria in a meso- to hyper-endemic form, primarily attributed to P. falciparum, owing to the region's distinct ecological factors [2]. These environmental features, such as hilly terrains, forest fringes, and foothill ecosystems, foster optimal conditions for malaria vectors, intensifying its prevalence and impact on public health. Early detection of malaria holds paramount importance in mitigating its consequential morbidity and mortality, especially given its effective treatment options if identified promptly. Clinical suspicion of malaria, particularly in cases of fever within endemic areas, is crucial for early intervention [3]. Notably, P. falciparum-induced malaria represents a medical emergency due to its potentially severe outcomes. Hence, accurate and timely species identification becomes imperative to administer appropriate and supportive therapies, particularly in cases of cerebral malaria, which exhibit alarmingly high case fatality rates in developing countries, ranging between 30-40% [4]. Traditionally, microscopic examination of blood smears remains the standard diagnostic method for detecting malarial parasites, requiring a robust healthcare system with operational microscopes, trained staff, and consistent access to reagents and quality control measures [4]. However, rural areas, where the majority of malaria cases occur, often lack access to such facilities, posing a significant challenge to timely diagnosis and treatment. In response to these limitations, the immunochromatographic test (ICT) emerges as a promising alternative. This test detects specific proteins—histidine-rich protein-2 (HRP-2) from asexual and young gametocytes of P. falciparum, alongside pan-specific aldolase released by all four malaria-causing species—offering a swift and efficient means of diagnosing malaria within a mere 10-15 minutes. Its simplicity enables even untrained personnel to conduct the test, thus addressing the diagnostic gaps prevalent in remote and under-resourced settings.

Early detection of Plasmodium falciparum parasites is pivotal in averting the progression of malaria-related complications and subsequent morbidity. Within our hospital setting, the 'Malascan' rapid diagnostic test (RDT) is readily accessible for inpatient use as per the directives of the hospital administration. This RDT kit is specifically designed to identify the histidine-rich protein-2 (HRP-II) specific to P. falciparum and the pan-specific aldolase, which is secreted by all four Plasmodium species, including P. falciparum [5].

The primary objective of this investigation was to compare the performance of the antibody-based 'Malascan' RDT with the conventional microscopy technique. This comparative analysis aimed to assess various parameters such as sensitivity, specificity, positive predictive value, and negative predictive value. Furthermore, the study aimed to evaluate the potential utility of this RDT in regions where the availability of quality microscopy for malaria diagnosis is limited. This exploration of the RDT's efficacy in such contexts aimed to highlight its possible role in facilitating accurate and timely malaria diagnosis in resource-constrained settings.

Materials and Methodology: In all clinically suspected cases of malaria rapid diagnostic test (Malascan) is done and simultaneously thick and thin smears are prepared and sent to malaria research laboratory of Ispat General hospital for microscopic examination before starting treatment.

Place of study: Department of Paediatrics, Ispat General Hospital, Rourkela, Odisha, India.

Study Design: Comparative study of rapid diagnostic test i.e. Malascan with gold standard routine microscopy and its validity in the form of sensitivity, specificity, positive predictive value, and negative predictive value – A hospital-based study.

Study subjects: Children up to 15 years of age admitted in the paediatrics ward or paediatric intensive care unit of Ispat General Hospital, Rourkela.

Duration of study: One year from Jan 2020 to Dec 2020

Sample size calculation: There is no exact way or formula to calculate sample size in this type of study which is a small hospital-based study and not a population-based study. The mean admissions of microscopy-positive malaria cases in the paediatric ward and paediatric intensive care unit in the last three years is 117. With a 90% confidence interval estimated sample size was 105.

Inclusion criteria: Children up to age of 15 years with acute onset of fever with or without one or more of the following:

1. Splenomegaly
2. Pallor
3. Jaundice
4. Convulsions/altered sensorium

Exclusion criteria:

1. Patient with a history of intake of any parenteral or oral medication for current illness except paracetamol(antipyretic) before admission in our hospital.
2. Any known case of anemia or jaundice like sickle cell disease, thalassemia, autoimmune hemolytic anemia, and acute hepatitis.

In this study, 119 patients were included suspected of malaria. In all, a rapid diagnostic test (Malascan) was done and simultaneously thick and thin smears were prepared and sent to the malaria research laboratory of Ispat General Hospital for microscopic examination before starting treatment.

Microscopy: Blood collected under strict aseptic conditions either from a finger prick or via EDTA venous puncture is utilized to create examination slides. For the thick smear, three drops of blood are swiftly combined with the edge of a spreader in 3-6 circular motions, forming a roughly 1cm diameter smear of optimal thickness—wherein newsprint is faintly visible. This thick film must dry flat and shielded from flies, dust, and excessive heat to isolate leucocytes and malarial parasites as the sole discernible components. To produce a thin smear, a separate clean slide acts as a spreader. With a blood drop on a flat surface, the spreader is used to encourage blood flow along its edge, maintaining a 45° angle to create a fine film. Ensure consistent contact between the spreader and the slide surface throughout the blood-spreading process. Thoroughly dry the thick film; in cases where urgent results are needed, expedite drying cautiously by fanning or gently exposing it to mild heat, being cautious not to overheat and inadvertently heat-fix the thick film. To fix the thin film, use three drops of methanol or immerse it briefly in a container of methanol. Avoid methanol exposure to the thick film to facilitate dehaemoglobinization.

Create a 10% Giemsa solution in either buffered or distilled water, ensuring the appropriate concentration by using three drops of stain per ml of buffered water.

Apply the stain meticulously, rinse with water drops to remove excess stain, and allow the slides to drain with the film-side down, taking care that the film does not make contact with the slide rack to prevent smudging. Examine these prepared smears under a compound microscope using a 100X oil immersion objective and a 10X eyepiece, meticulously counting a minimum of 200 leucocytes to identify the presence or absence of parasites. Positive smears undergo grading according to the plus system, while negative smears require a count of at least 500 leucocytes.

Regarding the Rapid Diagnostic Test (RDT):
Execute the RDT utilizing finger-prick blood to identify the HRP-2 antigen of P. falciparum and the pan-specific aldolase. The test card features a control antibody reaction zone along with two test lines—one specific for HRP-2 and the other for pan-specific aldolase, recognizing all four Plasmodium species. Employ the "Malascan" kit, adhering to the following steps:

1. Ensure that the components of the MALASCAN ICT kit are at room temperature before initiating the testing process.
2. Rotate the vial cap of the provided clearing buffer clockwise to facilitate the opening of the dropper bottle nozzle for subsequent use.
3. Collect the finger prick blood and gently touch the sample loop to the blood.
4. Ensure that a complete loop of blood is acquired, promptly transferring it to sample port 'A'. Take care to prevent clotting and ensure immediate transfer.
5. Without delay, add two drops of the clearing buffer into buffer port 'B' by holding the plastic dropper bottle in a vertical position.
6. Review the test outcomes precisely at the conclusion of a 20-minute period.

RDT is positive when: one or both of the test lines/reaction zones are positive along with control line.

RDT is negative when: only control line is positive and test lines are negative.

Results: Total malaria positive (i.e. microscopy positive) cases were 62 and malaria negative cases were 57. RDT positive cases 60 and RDT negative were 59.

Table 1

	Malaria positive	**Malaria negative**	
RDT positive	59	1	60
RDT negative	3	56	59
total	62	57	**119**

Statistical analysis was conducted using Fisher's exact test through the SPSS software, revealing a two-tailed p-value below 0.0001. This indicates a significant statistical association between the rows (groups) and columns (outcomes) in the dataset.

Table 2: Performance of RDT (malascan) in comparison to the gold standard – microscopy in diagnosing malaria

True positive (Both microscopy and RDT is positive)	59
True negative (Both microscopy and RDT is negative)	56
False positive (RDT is positive but microscopy negative)	1
False negative (RDT is negative but microscopy positive)	3
Sensitivity of RDT (malascan)	95.16%
Specificity of RDT (malascan)	98.24%
Positive predictive value	98.33%
Negative predictive value	94.91%

Our study has shown that "malascan" had excellent sensitivity (95.16%) and specificity (98.24%) for detecting malaria. Malascan test had a positive predictive value of 98.33% and, a negative predictive value of 94.91% with a p-value <0.0001.

Discussion:

The recent upsurge in malaria cases has sparked a renewed focus on developing rapid diagnostic techniques alongside preventive measures. Among these methods, serological immunochromatographic tests, including Malascan, have shown promise. In our evaluation involving 119 suspected malaria cases, microscopy detected 62 positive cases (59 falciparum, 6 vivax, 2 malariae, and 5 mixed infections), while Malascan identified 60 cases, missing detection in 3 instances. Among the confirmed malaria cases (62), 37 were male and 25 were female. Fever was present in all patients, while other common symptoms included splenomegaly (83.8%), pallor (82.2%), convulsions or altered sensorium (51.6%), and jaundice (22.5%). Our study showed sensitivity and specificity of **95.16%** and **98.24%** respectively for malascan which fulfils the WHO standard of minimum of 95% sensitivity and 95% specificity for a RDT to be used for diagnosis of malaria. The positive predictive value and negative predictive value of malascan are 94.91**%** and **98.33%** respectively**.**

In regions like ours, where both falciparum and vivax malaria prevail, there's a crucial need for an RDT capable of detecting and distinguishing between these two parasite species when they occur as single infections. Malascan's ability to identify falciparum-specific HRP-II and panspecific aldolase enables differentiation between falciparum and non-falciparum malaria. However, there are two limitations with this kit: it cannot distinguish falciparum malaria from mixed infections, and as vivax malaria is predominant among non-falciparum cases in our region, it often categorizes non-falciparum as vivax malaria.

However in our hospital which is situated in Rourkela, Sundergarh district where plasmodium falciparum is the predominant species (constitute 90% of malaria in the area and 95% of malaria admitted in our paediatrics department), malascan is useful RDT for early diagnosis of falciparum and therefore early institution of treatment. If there is mixed infection (Pf and pan both positive or only pan positive) or only non-falciparum malaria i.e. only pan positive (which is almost always vivax in our country) initial treatment is started as for falciparum and changed according to microscopy.

Conclusion:

Our research highlights "Malascan" as a straightforward and efficient bedside diagnostic test for malaria, boasting a sensitivity of 95.16% and specificity of 98.24%. Its operation doesn't necessitate highly specialized personnel for execution or result interpretation.

In settings like our hospital, where quality microscopy is accessible, RDTs can operate concurrently with microscopy. RDTs serve for swift or preliminary diagnosis, while microscopy remains reserved for complex cases or confirming negative RDT results with strong clinical suspicion of malaria. For non-falciparum malaria (pan positive in Malascan), treatment initially mirrors falciparum malaria (prevailing in over 90% of cases in our region), later adjusted based on microscopy findings. Consequently, Malascan facilitates immediate malaria confirmation even in peripheral healthcare setups lacking quality microscopy, administered by minimally trained personnel. Thoughtful Malascan usage alongside microscopy may offer various advantages:

1. Administering prompt treatment can lower both mortality and morbidity rates.
2. Costly medications and drug combinations in regions with multi-drug resistance will be reserved for individuals specifically requiring them.
3. Preventing unnecessary treatment will alleviate drug pressure and potentially slow the advancement of drug resistance.

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