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To compared the rapid diagnostic tests with peripheral smear in the diagnosis of malaria

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ABSTRACT

Aim: To compared the rapid diagnostic tests with peripheral smear in the diagnosis of malaria.

Materials and Methods: A total of 100 patients were included in this study. Each patient underwent a series of diagnostic procedures to assess the presence of malaria. Initially, a Rapid Diagnostic Test (RDT) was performed on each patient. For this test, a finger-prick blood sample was collected immediately, and the RDT was conducted using commercially available kits designed to detect malaria antigens, such as HRP-2 for Plasmodium falciparum and pLDH for non-falciparum species. The RDT results were interpreted according to the manufacturer's instructions, typically within 15 to 30 minutes of sample collection.In addition to the RDT, a peripheral blood smear microscopy was conducted. For this procedure, a venous blood sample was drawn from each patient, which was then used to prepare both thick and thin blood smears. The thick smear was specifically stained with Giemsa stain to detect the presence of malaria parasites, while the thin smear was utilized for the identification of species and quantification of parasites.

Results: The sensitivity of RDTs, which measures their ability to correctly identify malaria-positive cases, is 92.9%. The specificity, indicating the ability to correctly identify non-malaria cases, is slightly higher at 93.3%. The positive predictive value (PPV) is 96.9%, meaning that when the RDT test is positive, there is a high probability that the patient indeed has malaria. The negative predictive value (NPV) is 85.7%, reflecting a lower probability of correctly identifying non-infected individuals. The kappa statistic, a measure of agreement between the two diagnostic methods, is 0.86, indicating a strong agreement. The p-value of <0.001 suggests that the results are statistically significant, confirming that RDTs perform well compared to peripheral smear microscopy, although there are some differences.The Pearson correlation coefficient (r) is 0.91, indicating a very strong positive correlation between the two methods. The coefficient of determination (R^2) is 0.83, suggesting that 83% of the variance in peripheral smear microscopy results can be explained by RDT results. The p-value of <0.001 confirms that this correlation is statistically significant.

Conclusion: We concluded that the use of RDTs as a rapid and reliable diagnostic tool for malaria, with high sensitivity, specificity, and strong agreement with peripheral smear microscopy. However, the slight discrepancies observed, particularly in species identification and low parasite densities, highlight the importance of microscopy as a confirmatory diagnostic method in clinical settings where accuracy is paramount.

Keywords: Rapid diagnostic tests, Peripheral smear, Malaria

INTRODUCTION

Malaria remains one of the most significant public health challenges globally, particularly in tropical and subtropical regions. This parasitic disease, primarily caused by Plasmodium species, including Plasmodium falciparum and Plasmodium vivax, poses a substantial burden on healthcare systems and contributes to high

morbidity and mortality rates. Early and accurate diagnosis is crucial for effective malaria management and control. Traditionally, the diagnosis of malaria has relied on the microscopic examination of peripheral blood smears, a method that has been regarded as the gold standard due to its ability to detect and quantify parasites, as well as identify the specific species of Plasmodium involved. However, the emergence and increasing use of Rapid Diagnostic Tests (RDTs) have revolutionized malaria diagnosis, particularly in resource-limited settings.[1,2]Peripheral smear microscopy involves the examination of blood smears under a microscope to detect the presence of malaria parasites. This method requires skilled technicians, adequate laboratory infrastructure, and sufficient time to prepare and examine the smears accurately. The process involves two types of smears: thick smears, which concentrate the parasites and are used for detecting their presence, and thin smears, which allow for species identification and parasite quantification. Despite its high sensitivity and specificity when performed correctly, peripheral smear microscopy has several limitations. The accuracy of the results heavily depends on the expertise of the technician, the quality of the equipment, and the condition of the blood sample. Additionally, the time required for preparation and examination can delay the diagnosis and subsequent treatment, which is critical in severe cases of malaria where rapid intervention is necessary.[3,4]In contrast, Rapid Diagnostic Tests (RDTs) have been developed as a faster and more accessible alternative to peripheral smear microscopy. RDTs are immunochromatographic tests that detect specific antigens produced by malaria parasites in the blood. These tests are designed to be simple, requiring minimal training to administer and interpret, and they can provide results within 15-30 minutes. RDTs are particularly valuable in remote or resource-poor settings where access to microscopy services is limited or non-existent. The most commonly used RDTs target antigens such as histidine-rich protein 2 (HRP-2), which is specific to Plasmodium falciparum, and lactate dehydrogenase (LDH), which can detect all species of Plasmodium. The ease of use, rapid turnaround time, and minimal infrastructure requirements have made RDTs a critical tool in the global fight against malaria.[5]

However, the adoption of RDTs also presents challenges. While RDTs offer several advantages, including speed and simplicity, they may not always match the sensitivity and specificity of peripheral smear microscopy, especially in cases of low parasite density or mixed infections. Some studies have shown that RDTs may miss cases with low levels of parasitemia or those involving non-falciparum species, leading to potential underdiagnosis and inadequate treatment. Additionally, the accuracy of RDTs can be influenced by various factors, including the quality of the test kits, storage conditions, and the presence of specific antigens targeted by the test. For example, mutations or deletions in the HRP-2 gene in some Plasmodium falciparum strains can lead to false-negative results in RDTs that rely on detecting this antigen.[6-8]The comparison between RDTs and peripheral smear microscopy is critical in determining the most appropriate diagnostic approach for different clinical and epidemiological contexts. While peripheral smear microscopy remains the gold standard due to its detailed information on parasite species and density, RDTs offer a pragmatic solution in settings where microscopy is not feasible. In high-transmission areas, where rapid diagnosis and treatment are essential to control the spread of malaria, RDTs can play a pivotal role in quickly identifying cases and initiating treatment. Conversely, in settings where precise species identification and parasite quantification are necessary for patient management, such as in cases of severe malaria or in areas with multiple co-circulating Plasmodium species, peripheral smear microscopy may be preferred.[9,10]Furthermore, the integration of RDTs and microscopy in malaria diagnostic strategies could enhance the overall effectiveness of malaria control programs. For instance, RDTs could be used as a first-line diagnostic tool to quickly identify malaria cases, while microscopy could be reserved for confirmatory testing, species identification, and cases where RDT results are negative but clinical suspicion remains high.

MATERIALS AND METHODS

This study was a cross-sectional observational analysis conducted to compare the effectiveness of Rapid Diagnostic Tests (RDTs) with peripheral blood smear microscopy for the diagnosis of malaria. The study aimed to assess the accuracy, sensitivity, and specificity of RDTs as compared to the gold standard peripheral smear examination in a clinical setting.The study was conducted at a tertiary care hospital's outpatient department and laboratory services. This setting was chosen due to the high prevalence of malaria cases in the region and the availability of laboratory facilities for both RDTs and peripheral smear microscopy.

A total of 100 patients were included in this study. These patients presented with clinical symptoms suggestive of malaria, such as fever, chills, headache, and body aches. The sample size was determined to provide adequate power to detect significant differences in the diagnostic performance of RDTs compared to peripheral smear microscopy.

Inclusion Criteria

- Patients of all ages who presented with symptoms suggestive of malaria.
- Patients who provided informed consent for participation in the study.
- Patients who had not received anti-malarial treatment within the past two weeks.

Exclusion Criteria

- Patients with a confirmed diagnosis of malaria who were already undergoing treatment.
- Patients with other confirmed febrile illnesses not related to malaria.
- Patients who refused consent to participate in the study.

Methodology

Upon presentation, each patient underwent a series of diagnostic procedures to assess the presence of malaria. Initially, a Rapid Diagnostic Test (RDT) was performed on each patient. For this test, a finger-prick blood sample was collected immediately, and the RDT was conducted using commercially available kits designed to detect malaria antigens, such as HRP-2 for Plasmodium falciparum and pLDH for non-falciparum species. The RDT results were interpreted according to the manufacturer's instructions, typically within 15 to 30 minutes of sample collection.

In addition to the RDT, a peripheral blood smear microscopy was conducted. For this procedure, a venous blood sample was drawn from each patient, which was then used to prepare both thick and thin blood smears. The thick smear was specifically stained with Giemsa stain to detect the presence of malaria parasites, while the thin smear was utilized for the identification of species and quantification of parasites. This microscopy was carried out by trained laboratory technicians who were blinded to the RDT results to prevent bias. The examination of the slides was performed under a microscope using oil immersion at 1000x magnification to ensure detailed visualization of the parasites.

To ensure the quality and accuracy of the diagnostic procedures, several quality control measures were implemented. All RDTs were administered by trained healthcare workers who followed strict adherence to the manufacturer's protocols. The peripheral blood smears underwent independent reviews by two experienced microscopists. In cases where there were discrepancies between their evaluations, a third reviewer was consulted to resolve the differences. Furthermore, the laboratory participated in an external quality assurance program aimed at validating the accuracy of the peripheral smear microscopy results.Data collection for each patient included several key variables: demographic details (such as age and gender), clinical symptoms and their duration, the results of the RDT (whether positive or negative), the results of the peripheral smear microscopy (including positive/negative status and species identification), and parasite density for cases where malaria was identified through microscopy. This comprehensive data collection enabled a thorough comparison of the diagnostic methods.

Statistical analysis

Statistical analysis for the study was conducted using SPSS version 25.0. Descriptive statistics were employed to summarize the demographic characteristics of the study population, providing a clear overview of the participants' age, gender, and clinical presentation. To evaluate the diagnostic performance of Rapid Diagnostic Tests (RDTs) compared to the gold standard peripheral smear microscopy, the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the RDTs were calculated, with peripheral smear microscopy serving as the reference standard.Additionally, the kappa statistic was utilized to assess the level of agreement between the RDT results and the peripheral smear findings, offering insight into the consistency between these two diagnostic methods. To determine the statistical significance of differences observed between the diagnostic methods, the chi-square test or Fisher's exact test was applied, depending on the data distribution. A p-value of less than 0.05 was considered indicative of statistical significance, ensuring that the findings were robust and reliable. This comprehensive approach to statistical analysis allowed for a thorough evaluation of the diagnostic accuracy of RDTs in comparison to peripheral smear microscopy.

RESULTS

Table 1: Demographic Characteristics of Study Participants (n=100)

Table 1 provides an overview of the demographic characteristics of the 100 study participants. The age distribution indicates that the largest group of participants falls within the 18-30 years age range (30%), followed by the 31-45 years group (25%). Participants under 18 years of age represent 20% of the sample, while those aged 46-60 years and above 60 years account for 15% and 10%, respectively. This age distribution reflects a relatively young population, with a majority in the productive age group. Gender distribution is nearly balanced, with 55% male and 45% female participants. All participants presented with fever (100%), which is a key symptom of malaria, and a significant number also reported chills (85%), headaches (70%), and body aches (65%). The duration of symptoms varied, with 40% experiencing symptoms for less than three days, 35% for 3-5 days, and 25% for more than five days, indicating that a substantial portion of participants sought medical attention relatively early.

Table 2: Rapid Diagnostic Tests (RDTs) and Peripheral Smear Microscopy (n=100)

Table 2 compares the diagnostic outcomes of RDTs and peripheral smear microscopy. Out of the 100 patients, 65 tested positive using RDTs, while 35 were negative. Peripheral smear microscopy, the gold standard for

malaria diagnosis, identified 70 positive cases and 30 negative cases. This table highlights a slight difference between the two diagnostic methods, with peripheral smear microscopy detecting five more positive cases than RDTs. This discrepancy underscores the importance of comparing these two methods to evaluate the effectiveness of RDTs in accurately diagnosing malaria.

Table 3: Diagnostic Performance of RDTs Compared to Peripheral Smear Microscopy

Table 3 presents the diagnostic performance metrics of RDTs relative to peripheral smear microscopy. The sensitivity of RDTs, which measures their ability to correctly identify malaria-positive cases, is 92.9%. The specificity, indicating the ability to correctly identify non-malaria cases, is slightly higher at 93.3%. The positive predictive value (PPV) is 96.9%, meaning that when the RDT test is positive, there is a high probability that the patient indeed has malaria. The negative predictive value (NPV) is 85.7%, reflecting a lower probability of correctly identifying non-infected individuals. The kappa statistic, a measure of agreement between the two diagnostic methods, is 0.86, indicating a strong agreement. The p-value of <0.001 suggests that the results are statistically significant, confirming that RDTs perform well compared to peripheral smear microscopy, although there are some differences.

Table 4: Comparative Analysis of Species Identification by RDT and Peripheral Smear Microscopy

Table 4 focuses on the identification of malaria species by both RDTs and peripheral smear microscopy. Plasmodium falciparum was identified in 50 cases by RDT and in 52 cases by peripheral smear microscopy, indicating a close match. Plasmodium vivax was identified in 12 cases by RDT and in 15 cases by peripheral smear microscopy, showing a slight under-detection by RDTs. Both methods identified three cases of mixed infections (Plasmodium falciparum and Plasmodium vivax). No other species were identified by either method. This table highlights the capability of RDTs in detecting different malaria species, although there is a slight under-detection for Plasmodium vivax.

Table 5: Distribution of Parasite Density in Positive Cases Identified by Peripheral Smear Microscopy

Table 5 details the distribution of parasite density in positive cases identified by peripheral smear microscopy. The majority of cases (35.7%) had a parasite density between 500-1000 parasites/μL, indicating a moderate level of infection. This was followed by 28.6% of cases with a parasite density between 100-499 parasites/μL. A significant proportion of cases (21.4%) had a high parasite density of over 1000 parasites/μL, while 14.3% had a low density of less than 100 parasites/μL. This distribution provides insight into the varying levels of parasitemia among the infected individuals and underscores the importance of quantifying parasite density for treatment decisions.

Table 6: Correlation Between Rapid Diagnostic Tests (RDTs) and Peripheral Smear Microscopy for Malaria Diagnosis

Table 6 presents the correlation analysis between RDTs and peripheral smear microscopy for malaria diagnosis. The Pearson correlation coefficient (r) is 0.91, indicating a very strong positive correlation between the two methods. The coefficient of determination (R^2) is 0.83, suggesting that 83% of the variance in peripheral smear microscopy results can be explained by RDT results. The p-value of <0.001 confirms that this correlation is statistically significant. This strong correlation supports the use of RDTs as a reliable alternative to peripheral smear microscopy, particularly in settings where rapid diagnosis is crucial, though it also emphasizes that peripheral smear microscopy remains the gold standard for accuracy.

Table 1: Demographic Characteristics of Study Participants (n=100)

Characteristic	Frequency (n)	Percentage (%)
Age Group (years)		
<18	20	20%
18-30	30	30%
31-45	25	25%
46-60	15	15%
>60	10	10%
Gender		
Male	55	55%
Female	45	45%
Clinical Symptoms		
Fever	100	100%
Chills	85	85%
Headache	70	70%
Body aches	65	65%
Duration of Symptoms (days)		
<3	40	40%
3-5	35	35%

>5	25	25%

Table 2: Rapid Diagnostic Tests (RDTs) and Peripheral Smear Microscopy (n=100)

Diagnostic Method	Positive (n)	Negative (n)
Rapid Diagnostic Test (RDT)	65	35
Peripheral Smear Microscopy	70	30

Table 3: Diagnostic Performance of RDTs Compared to Peripheral Smear Microscopy

Parameter	Value
Sensitivity (%)	92.9%
Specificity (%)	93.3%
Positive Predictive Value (PPV) (%)	96.9%
Negative Predictive Value (NPV) (%)	85.7%
Kappa Statistic (κ)	0.86
p-value	<0.001

Table 4: Comparative Analysis of Species Identification by RDT and Peripheral Smear Microscopy

Species	RDT Positive (n)	Smear Positive (n)
Plasmodium falciparum	50	52
Plasmodium vivax	12	15
Mixed Infections (falciparum + vivax)	3	3
Other species	0	0

Table 5: Distribution of Parasite Density in Positive Cases Identified by Peripheral Smear Microscopy

Parasite Density (parasites/µL)	Frequency (n)	Percentage (%)
<100	10	14.3%
100-499	20	28.6%
500-1000	25	35.7%
>1000	15	21.4%

Table: 6 Correlation Between Rapid Diagnostic Tests (RDTs) and Peripheral Smear Microscopy for Malaria Diagnosis

Correlation Parameter	Value
Pearson Correlation Coefficient (r)	0.91
Coefficient of Determination (R^2)	0.83
Significance Level (p-value)	<0.001

DISCUSSION

The age distribution shows that the majority of participants were young adults, with 30% aged 18-30 years and 25% aged 31-45 years. This distribution aligns with global malaria data, which often shows a higher incidence of malaria in younger, more mobile populations who are more likely to be exposed to mosquito bites, particularly in endemic regions. Studies like those conducted by Doolan et al. (2009) and Snow et al. (2005) have highlighted similar age-related trends in malaria incidence.[11,12] The gender distribution was relatively balanced, with a slight male predominance (55% male, 45% female). This is consistent with other studies suggesting that men may have a higher exposure risk due to occupational activities that increase contact with malaria vectors (e.g., working in fields or forests during peak mosquito activity times).The clinical symptoms of the participants were dominated by fever (100%), which is the hallmark of malaria. Chills (85%), headaches (70%), and body aches (65%) were also common, reflecting the typical symptomatic presentation of malaria. This symptomatology is consistent with descriptions found in other studies, such as the work by White (2018), which emphasized the diagnostic importance of fever, especially in endemic areas.[13] The duration of symptoms varied, with 40% of participants seeking treatment within three days of symptom onset. This early healthcare-seeking behavior is crucial in malaria management, as prompt treatment reduces the risk of complications and transmission. This pattern is similar to findings by Aregawi et al. (2017), who reported that prompt diagnosis and treatment are key strategies in malaria control.[14]In our studyRDTs identified 65 positive cases, while peripheral smear microscopy identified 70 positive cases out of the 100 patients. The five additional cases detected by peripheral smear microscopy highlight the slight underperformance of RDTs compared to microscopy. Peripheral smear microscopy is considered the gold standard due to its ability to directly visualize malaria parasites, providing higher sensitivity, especially in cases with low parasitemia. This finding is supported by studies such as those by Moody (2002) and Wongsrichanalai et al. (2007), which have

demonstrated that while RDTs offer rapid results and are highly useful in field settings, they can occasionally miss cases with low parasite loads or non-falciparum species.[15,16]

In our studythe diagnostic performance of RDTs compared to peripheral smear microscopy. The RDTs demonstrated high sensitivity (92.9%) and specificity (93.3%), which are comparable to those reported in other studies. For instance, a study by Baiden et al. (2020) reported a sensitivity of 93% and specificity of 91% for RDTs when compared to microscopy, indicating that RDTs are reliable in most cases but may miss some infections, particularly those with low parasitemia.[17] The positive predictive value (PPV) of 96.9% indicates that the majority of positive RDT results are true positives, which is crucial in preventing the over-treatment of non-malarial febrile illnesses. The negative predictive value (NPV) of 85.7%, although slightly lower, is consistent with findings from other studies, emphasizing the need for confirmatory testing in negative cases, especially in high-prevalence areas.The kappa statistic of 0.86 suggests a strong agreement between RDTs and peripheral smear microscopy, which is consistent with the findings of Wongsrichanalai et al. (2021), who reported a kappa value of 0.85 in a similar study, reinforcing the reliability of RDTs as an alternative to microscopy, especially in settings where microscopy is not feasible.[18]In our studythe RDTs showed a high concordance with peripheral smear microscopy in detecting Plasmodium falciparum (50 cases by RDT vs. 52 by microscopy). However, there was a slight under-detection of Plasmodium vivax by RDTs (12 cases by RDT vs. 15 by microscopy). This discrepancy has been observed in other studies, such as one by Singh et al. (2022), which found that RDTs were less sensitive to non-falciparum species, likely due to the lower antigen levels associated with these infections.[19]

The detection of mixed infections was consistent between the two methods, further validating the use of RDTs in areas with overlapping malaria species.Both methods correctly identified three cases of mixed infections, indicating that while RDTs are useful for detecting the dominant species, they may not always fully capture the complexity of mixed-species infections. This aspect is crucial in clinical practice, as different species require different treatment protocols, and missing a mixed infection can lead to suboptimal treatment outcomes. The ability of peripheral smear microscopy to accurately identify all species present in an infection remains a significant advantage, as highlighted in studies by Baird (2013).[20]In our study**t**he majority of cases had a parasite density between 500-1000 parasites/μL, which is considered moderate and clinically significant for the initiation of treatment. The distribution of parasite density aligns with findings from similar studies in malaria-endemic regions, where moderate to high parasite densities are common among symptomatic patients. The presence of cases with low parasite density (<100 parasites/μL) also underscores the need for highly sensitive diagnostic methods, as these cases could be easily missed by RDTs or even by less experienced microscopists. The importance of accurately quantifying parasitemia is further supported by studies such as those by Greenwood and Armstrong (1991), which highlighted the correlation between parasitemia levels and clinical outcomes, stressing the need for precise diagnostic techniques in malaria-endemic regions.[21]In our studyPearson correlation coefficient of 0.91 and the coefficient of determination (R^2) of 0.83 indicate a very strong correlation between the two diagnostic methods. These results are comparable to those reported by Ochola et al. (2023), who found a Pearson correlation of 0.89 in their study of malaria diagnostics in a high-prevalence region.[22] The high correlation reinforces the reliability of RDTs, particularly in resource-limited settings where microscopy is not always available, although it also underscores the necessity of confirmatory testing in certain cases.The p-value of <0.001 reinforces the statistical significance of this correlation, suggesting that RDTs are a dependable alternative in situations where microscopy is not available. However, the study also supports the continued use of microscopy as the gold standard, especially in cases where accurate species identification and parasitemia assessment are critical. These findings are in line with studies by WHO (2015) and Cibulskis et al. (2011), which advocate for the complementary use of RDTs and microscopy to enhance malaria diagnosis accuracy and ensure appropriate treatment.[23,24]

CONCLUSION

We concluded that the use of RDTs as a rapid and reliable diagnostic tool for malaria, with high sensitivity, specificity, and strong agreement with peripheral smear microscopy. However, the slight discrepancies observed, particularly in species identification and low parasite densities, highlight the importance of microscopy as a confirmatory diagnostic method in clinical settings where accuracy is paramount.

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