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COMPARATIVE EFFICACY OF RAPID DIAGNOSTIC TESTING VS MICROSCOPY FOR DIAGNOSING MALARIA

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ABSTRACT

Background: *The different species of Plasmodium are the causative agents of malaria, and it is an obligate intracellular parasite spread by the bite of an infected female Anopheles mosquito. In underdeveloped nations, malaria causes high rates of morbidity and mortality, making it a major public health problem.*

Objective: *This study was conducted to compare the efficacy of Rapid Diagnostic Test verses Microscopy for malarial diagnosis.*

Materials and Methods: *This cross-sectional study was conducted at Bacha khan medical college Mardan from April 2024 to December 2024 to compare the efficacy of Rapid Diagnostic Testing Vs Microscopy for malarial diagnosis. A total of 150 blood samples from suspected malaria patients were included in our study. Their blood samples were aseptically drawn and dispensed into an EDTA container for RDTs and microscopic analysis. All the data was analyzed by using SPSS version 24.*

Results: *This study included 150 patients based on the inclusion criteria. Of them, 80 (53.3%) were male and 70 (46.6%) were female. 100 patients (66.6%) had positive microscopy results for malaria, 40 individuals (26.6%) had positive RDT results, and 50 individuals (3.3%) who had negative microscopy results also had negative RDT results.*

Conclusion: *This study demonstrates that malaria can be diagnosed more accurately using microscopy. Importantly, a negative malaria test result by RDT may not always indicate absence of malaria. According to our study, microscopy is the gold standard in diagnosing malaria when compared to RDT.*

Keywords: *RDT, Microcopy, Malaria, Diagnosis.*

INTRODUCTION

The different species of Plasmodium are the causative agents of malaria, and it is an obligate intracellular parasite spread by the bite of an infected female Anopheles mosquito [1]. In underdeveloped nations, malaria causes high rates of illness and mortality, making it a major public health problem [2]. Numerous Plasmodium species, including Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae, Plasmodium ovale, and Plasmodium knowlesi, are the cause of illness and are spread by female Anopheles mosquitoes [3]. The disease still poses a major hazard to global public health, even with significant achievements in lowering its morbidity and death [4]. Over 90% of the world's malaria cases occur in sub-Saharan Africa, making it a serious public health concern [5]. Plasmodium falciparum is the most lethal of the five species of malaria parasites (Plasmodium species) that infect humans because of its capacity to disrupt the host's physiology when it is developing in the blood stages [6]. The primary approach to controlling malaria is prompt, precise diagnosis followed by efficient treatment [7]. In particular, more than 95% of all cases and fatalities occur in Africa, making it the most impacted continent [8]. Globally, 610,000 people died from malaria in 2021, and 244 million cases were documented [8]. Both efficient health care and malaria surveillance depend on early and precise diagnosis. In every environment, accurate malaria diagnosis is crucial since incorrect diagnoses can lead to serious morbidity and mortality. The WHO has advised since 2010 that prior to receiving treatment, all patients with suspected malaria should have their diagnosis verified by microscopy or a rapid diagnostic test (RDT) [9]. Only 62% of patients suspected of having malaria had received a diagnostic test, according to a World Health Organization (WHO) report [10]. Before beginning therapy, the World Health Organization (WHO) advises all patients with suspected malaria to have a rapid diagnostic test (RDT) or parasitological confirmation by microscopy [11,12]. Just in cases where a parasitological and/or RDT diagnosis is not available, treatment should be done based simply on clinical suspicion [13,14]. The two main methods for diagnosing malaria in the field are microscopy and RDTs. False-negative results, on the other hand, have the potential to postpone treatment and expand the population of mosquito-infecting individuals. In nations where malaria is endemic, microscopy remains the "gold standard" for diagnosing the disease. The sensitivity of this technique ranges from 50 to 500 parasites/µl [15] is affordable and enables the detection of parasite density and species [16, 17]. A lack of qualified microscopists, poor quality control, and the potential for misinterpretation due to low parasitaemia or mixed infections are some of the drawbacks of microscopic diagnosis in many malaria-endemic areas [18,19]. Predictive diagnosis is still commonly utilized, and some rural health facilities do not have access to this diagnostic technique [20,21]. Furthermore, some Plasmodium species are not reported in the country because it can be challenging to identify them using microscopy. For example, Plasmodium ovale shares a morphology with P. vivax, but this has significant implications for malaria epidemiology and mapping even if it has no bearing on treatment because the patient will receive the same treatment for both species [22]. Rapid Diagnostic Tests (RDTs) are immune-chromatographic diagnostic tests that are used to diagnose or confirm malaria parasitologically in remote areas. The most common RDTs for malaria are based on the detection of parasite histidine-rich protein II (HRP2). These Point of Care (PoC) tests are quick, inexpensive, simple to use, and straightforward to interpret; results are available in a matter of minutes, and they don't require a lot of staff or electrical equipment. False positive or negative results are the main limitation of RDTs. While RDT-negative but microscopy-positive results can be caused by operator error, poor storage conditions, deletions in the P. falciparum histidine-rich protein 2 and 3 genes, and inadequate efficacy of particular RDT brands, false positives happen because HRP2 remains in the blood for several days after infection clearance [23]. Therefore, using extremely accurate molecular techniques to appropriately assess the effectiveness of such diagnostic tests is essential [24].

Materials and Methods

This cross-sectional study was conducted at Bacha khan medical college Mardan from April 2024 to December 2024 to compare the efficacy of Rapid Diagnostic Testing Vs Microscopy for malarial diagnosis. A total of 150 blood samples from suspected malaria patients were included in our study. Their blood samples were aseptically drawn and dispensed into an EDTA container for RDTs and microscopic analysis. Within 10 minutes of collection, thick and thin films were created in triplicate from EDTA samples, and as

soon as the clots were completely formed, sera were extracted from the plain tubes. Giemsa's and Field's methods were used to stain the thick films, while Lieshman's and diluted Giemsa's methods were used to stain the thin films. RDT based on antigens was performed on duplicate aliquots of hemolyzed whole blood, and sera were tested in duplicates to detect antibodies to malaria parasites based on RDT's antibody detection methods. Patients who refused to participate were excluded from the study, as well as those who had taken anti-malaria medications or herbal remedies within the two weeks prior to, or who had a severe clinical condition requiring immediate medical attention. The data was analyzed by using SPSS version 24.

Results

This study included 150 patients based on the inclusion criteria. Of them, 80 (53.3%) were male and 70 (46.6%) were female. The age range covered by the group was >15 to 40 [Table 1]. 100 patients (66.6%) had positive microscopy results for malaria, 40 individuals (26.6%) had positive RDT results, and 50 individuals (3.3%) who had negative microscopy results also had negative RDT results [Table 2].

Table 1: Sex and Age Distribution of the Patients

Sex	Frequency	Percentages
Female	70	46.6%
Male	80	53.3%
Total	150	100%
Age groups		
16-22 years	62	41.3%
23-35 years	50	33.3%
36-40 years	38	25.3%
Total	150	100%

Table2: Comparison between Microscopy and Rapid Diagnostic Test

	Microscopy n (%)	RDT n (%)
Total patients	150	150
Positive	100 (66.6%)	40 (26.6%)
Negative	50 (33.3%)	110 (73.3%)
Percentage of positive	66.6 %	26.6%

Discussion

In endemic areas where the four species of malaria parasites are present, an accurate diagnosis of Plasmodium species is crucial for both developing the appropriate treatment plan and implementing successful malaria control measures. When Plasmodium species are misidentified, improper therapies may cause regrowth and even drug resistance, which could pose serious public health risks [25]. In order to control malaria, a high-quality diagnostic technique is needed to identify the parasite before anti-malarial medication is prescribed in accordance with WHO guidelines. Early parasite identification, therapy targeting, and treatment response characterization are all made possible by malaria parasitological diagnosis [26]. The performance of two common malaria diagnostic techniques—the Rapid Diagnostic Test (RDT) and the microscope technique—was examined in this study. Over half of the participants were between the ages of 16 and 23. This proved that younger people had a higher rate of transmission than older people. This is consistent with a research by Enitan et al. [27] et al. that found that individuals between the ages of 16 and 25 had a greater prevalence of P. falciparum malaria infection. In a related study, Adesina [28] found that students at the University of Maiduguri in northeastern Nigeria who were between the ages of 17 and 19 had a greater prevalence of malaria. Using thick blood films stained with Field stain A and B, we evaluated the SD Bioline test kit's performance and the use of microscopy in this investigation. When compared to microscopy, we observed that the SD Bioline test kit produced poor test findings. It's probable that SD

Bioline missed some malaria infections that were identified by blood films. It's also possible that some participants were already taking medication when their symptoms first started and didn't disclose this to the health centers. Additional factors that could impact the RDT's stability, such as extremely high or low temperatures that could compromise the test's effectiveness during storage and transit, could be the cause of the low positive rates [29]. The low positive results may also be caused by high humidity, which has been reported to degrade RDTs [30].This study has demonstrated that SD Bioline Malaria Ag P.f is easy to use and the results were ready in 12 minutes. Our findings support the ability of this RDT to detect parasite antigens (Histidine Rich Protein-II) from finger prick blood, allowing efficient handling for non-technician with less training [31]. The use of microscopy, however, gave better (66.6%) results than use of RDT, which is similar to those obtained by Azikwe et al. (2012) [32], Harcut et al. (2013) [33], and Elechi et al. (2015) [34]. This low sensitivity will hinder control intervention because a portion of the infected population will go untreated, particularly if RDT is the only diagnostic method available. The sensitivity reported in this study falls short of the 95% recommended World Health Organization value [35]. This might have significant effects on mortality, transmission, and health. This study's RDT sensitivity is lower than that of earlier studies conducted in Zambia, Zanzibar, Nigeria [36], and Thailand [37, 38, 39]. Crucially, the current study found that the RDT had 100% specificity, which is significantly greater than the Burkina Faso report [40] but comparable to the findings among the isolates from Nigeria [41]. The RDT employed in this study may be able to identify more negative instances and fewer positive ones than the microscopic test. Lower positive rates may be caused by outside variables that could compromise the RDT's stability, according to [42]. Among these are exposure to extremely high or low temperatures, which has been shown to be a major influence in the poor performance of quick diagnostic tests, especially while they are being transported and stored.

Conclusion

This study demonstrates that malaria can be diagnosed more accurately using microscopy. Importantly, a negative malaria test result by RDT may not always indicate absence of malaria. According to our study, microscopy is the gold standard in diagnosing malaria when compared to RDT. In rural areas, RDT can still be used as the initial screening test for the diagnosis of malaria but for the diagnosis of malaria, we advise using microscopy as much as possible after RDT

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