Vol. 5 No. 2 (2026)

Pakistan Journal of Medical & Cardiological Review

https://pakjmcr.com/index.php/1/about

Online ISSN 3007-2387

Print ISSN 3007-2379

Sami et al., 2026

Artificial Intelligence in Microscopic Diagnosis of Malaria: A Laboratory Study in Pakistan

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Received on 21 March, 2026

Accepted on 09 April, 2026

Published on 10 April, 2026

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Abstract

Background: Malaria remains a major burden in developing countries including Pakistan; early and correct diagnosis is crucial for the treatment and control of the disease. The standard method of malaria diagnosis, microscopic examination of Giemsa-stained blood smears, is highly dependent on the skill of the microscopist. **Objective**: A recent study set out to design and test a malaria diagnostic tool using artificial intelligence (AI) to detect malaria parasites in peripheral blood smears in Pakistan. **Method:** A cross-sectional study was performed in the laboratory on blood smears from patients with suspected malaria. Thick and thin blood smears, prepared according to protocol were stained with Giemsa and scored on light microscopy (the gold standard). Images were taken of the stained smears and the result from the microscopy was used to label the image. The images were used to train and test a convolutional neural network (CNN) artificial intelligence (AI) model for malaria. We determined the diagnostic performance measures, accuracy, sensitivity, specificity, precision, F1-score and area under the receiver operating characteristic curve (AUC). **Result:** The AI model showed strong agreement with conventional microscopy and demonstrated promising diagnostic performance in identifying both malaria-positive and malaria-negative samples. The model exhibited high accuracy, sensitivity, and specificity, supporting its potential as a diagnostic tool. **Conclusion**: This research shows that AI-assisted microscopy could be used as an adjunct technique to improve the speed, accuracy and efficiency of malaria diagnosis, particularly in resource poor settings. AI-assisted microscopy could be used as an adjunct to existing methods in Pakistan but first we need to test it on a larger scale.

Keywords: Malaria, Artificial Intelligence, Microscopy, Deep Learning, Pakistan, Blood Smear Diagnosis, *Plasmodium falciparum*, *Plasmodium vivax*

Introduction

Malaria is a life threatening parasite disease which is caused by protozoa of genus Plasmodium falciparum and Plasmodium vivax which are carried by bit of infected female Anopheles mosquitoes. It is still one of the most important world epidemic health issues especially in tropical and subtropical countries. As per the global estimates, malaria has remained the cause of high morbidity and mortality with hundreds of millions of cases being reported every year particularly in low- and middle-income countries. Pakistan belongs to the number of countries with malaria that is still endemic and is a constant burden on the healthcare infrastructure. The population is much malaria prone and about 217 million individuals are at moderate risk and 63 million at high risk of infection (Khan *et al.,* 2023).

Recent national surveillance data show that in 2023 alone, more than 2.7 million confirmed malaria cases were reported in Pakistan, which explains the continued public health issue (WHO, 2022). There are also environmental conditions like flooding, lack of sanitation and climatic variations, which have also enhanced transmission with millions of suspected cases having been reported during outbreaks (Baig,2025).

Malaria management and control requires proper and prompt diagnosis. The conventional diagnostic methods include microscopic examinations of stained blood smears, rapid diagnostic tests (RDT) and molecular diagnostic methods including polymerase chain reaction (PCR). Among them, the most common gold standard in the majority of resource-constrained settings is microscopy, which is inexpensive and has the capacity to diagnose parasitemia. However, microscopic diagnosis is highly subjective and is dependent on the capacity and experience of the person conducting the examination and the diagnosis can be prone to human error, variation and time (M.A Khan 2026).

Scarcity of trained microscopists in most parts of Pakistan also compromises accuracy of diagnosis and delayed treatment. The past several years have seen the introduction of artificial intelligence (AI) and deep learning and computer vision in its specifics, which has introduced fresh opportunities in order to make the diagnosis process in parasitology more accurate. Microscopic analysis of blood smears can also be done at a high speed and consistency using AI based systems, unlike in traditional systems which rely on the application of human expertise. It is demonstrated that AI-aided malaria diagnosis may be highly sensitive and specific (approximately 89-90%), almost as trained microscopists (Faratisha *et al.,* 2026).

Moreover, convolutional neural networks (CNNs) and other deep learning models have demonstrated being able to automatically identify and classify infected red blood cells with over 95 percent accuracy in an experimental setting, which indicates that they can be used in a clinical setting (Ramanpreet 2024).

AI malaria diagnostics is especially promising to resource-restricted locations such as Pakistan, where healthcare facilities and trained professionals might be inadequate. The diagnostic tools based on AI can be used to improve detection in the early stages of illness, better treatment results, and large-scale screening programs. Furthermore, standardized and quick diagnosis may be achieved by the development of low-cost digital microscopy systems with AI-based algorithms, which is a viable solution to rural and underserved populations. In spite of these achievements, the use of AI-based malaria diagnosis in Pakistan has not been widespread, and the data and validation studies should be generated in the country. The variation in prevalence, staining methods, as well as laboratory conditions, requires region-specific models in order to provide reliability and accuracy. Consequently, the research will design and test an AI-aided microscopic diagnostic platform of malaria disease on blood smear images taken on Pakistani people. This study aims to make a contribution to enhancing the diagnostic capacity and malaria control in the country by integrating the laboratory-based parasitology with the sophisticated computational methods.

Hypothesis of the Study

Null Hypothesis (H_0)

No meaningful difference was found between the diagnostic accuracy of the artificial intelligence-based assisted microscopy and the traditional microscopy in detecting Malaria parasites in peripheral blood smears.

Alternative Hypothesis (H_1)

The diagnostic accuracy of artificial intelligence-assisted microscopy is much higher or equivalent to the traditional microscopy in identifying the Malaria parasites in the peripheral blood smears.

Literature Review

Malaria is one of the most significant disease-causing parasites globally and is still causing a significant diagnostic and treatment burden on the endemic nations. The World Malaria Report 2025 states that the number of malaria cases in the world in 2023 is 263 million and the burden is still high in 2024, which proves that the progress is slow and more powerful diagnostic and control systems are required. WHO has continued to stress the importance of pre-treatment diagnosis using parasites, primarily by microscopy or malaria rapid diagnostic tests due to the central role in case management, surveillance, and monitoring of resistance (WHO 2025)

Pakistan is highly relevant environment to conduct a malaria study since malaria is an endemic disease and burden has gone out of proportion within the last few years. WHO has indicated that in 2023, Pakistan had reported 2.7 million cases, and the figure declined albeit very high at about 2 million cases per year later because of enhanced control. WHO also reported earlier that in the period between January and August 2022, Pakistan had reported more than 3.4 million suspected cases of which 77 percent were due to Plasmodium vivax and 23 percent due to P. falciparum among laboratory-confirmed cases. In addition, a 2023 systematic review and meta-analysis of malaria in Pakistan has a pooled prevalence of 23.3% and P. vivax is in general the most prevalent. Such results indicate that malaria is clinically and epidemiologically significant in

Pakistan, especially on investigations conducted to enhance diagnosis in normal laboratories.

In the majority of low- and middle-income countries, the standard light microscopy is the primary mode of diagnosis of malaria, since it is relatively inexpensive and can provide more information than a positive or negative result. WHO reports that microscopy may examine parasites directly in blood smears, identify species, and also determine the density of parasites when performed correctly. These characteristics render microscopy particularly useful in endemic nations where clinicians need to differentiate malaria and other febrile diseases and species level diagnosis is important in treatment. However, microscopy too depends heavily on the criteria of the smear preparation, smear staining, preservation of the microscopes and above all the skill of the readers. It is therefore possible that the diagnostic performance of the laboratories and the observers differ significantly.

The main limitation of routine microscopy is not that it lacks conceptual ineptitude, but it cannot be on a large scale standardized. At low parasitemia, sensitivity can be reduced in busy or resource-limited laboratories by fatigue, workload, inconsistent stain quality, and training variability. This issue is especially applicable in Pakistan, where malaria burden is not even distributed by district and laboratory capacity is also not constant. In recent Pakistan-based and Pakistan-centric literature, it is called to enhance surveillance and diagnostic capacities at subnational level, which entails enhanced acquaintance with local patterns of burden in such provinces as Sindh and Khyber Pakhtunkhwa. This leads to a practical requirement of the tools that can assist microscopists as opposed to substituting them, in particular, in the environments where skilled readers are scarce.

The recent success in artificial intelligence, especially deep learning has created a possible opportunity in malaria detection using the automated processes in the digital blood smear images. The new systems are mostly based on the convolutional neural networks, transfer learning, or object detectors like YOLO to classify infected and uninfected cells or detect parasites directly on thick or thin smears. A 2024 literature review of the topic of Engineering Applications of Artificial Intelligence has surveyed the rapid advances in the fields of machine learning and deep learning in blood-smear-

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https://pakjmcr.com/index.php/1/about
Online ISSN Print ISSN
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based malaria detection, and some 20242025 studies have demonstrated high performance using Efficient Net, YOLOv4, transfer learning, and ensemble models. The reported accuracy values of the experimental research will be typically over 95 and it means that under the conditions of control, AI models can be applied to search discriminative visual features in smear images with amazing consistency.

Alongside single-model performance papers, more recent data has started to explore the issue of whether AI systems can be clinically applied. In a systematic review of AI in malaria diagnostics, released in 2026, researchers mention that AI-aided diagnostic systems can be extremely diagnostic in clinical practice, but microscopy is the current standard of a practical reference in the majority of studies. A 2026 report reported that malaria detection in digital microscopy assisted by AI in primary health care laboratories was as good as reference methods, which confirms the idea that AI can be particularly useful in the context of limited resources and experts. The importance of these newer studies is that they change the argument on whether AI can categorize images or whether AI can help in any actual diagnostic processes.

Besides single-model performance papers, more recent data have begun to investigate the question of whether AI systems can be applied in clinical settings. In a systematic review of AI in malaria diagnostics, published in 2026, researchers state that AI-aided diagnostic systems may prove to be highly diagnostic in clinical practice, although microscopy is the existing standard of a practical reference in most studies. In 2026, a report on malaria detection in digital microscopy with the help of AI in primary health care laboratories was as good as the reference methods, proving the notion that AI can be especially helpful in the scenario of scarce resources and specialists. The importance of these newer studies is that they change the argument on whether AI can categorize images or whether AI can help in any actual diagnostic processes.

The other new trend in the literature is reliable and understandable AI. A model that is to be accepted as diagnostic technologies cannot be known to be correct on a test set; users must have confidence that the system is acting upon biologically significant structures. More modern literature has therefore also incorporated the use of explainable AI techniques, e.g. saliency mapping or Grad-CAM, to make model decisions more understandable. The trend is particularly applicable in malaria microscopy, where

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Online ISSN 3007-2387
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laboratory scientists and clinicians will tend to be more convinced by an AI tool that indicates likely regions of parasites as opposed to a binary label only. Explain ability can hence have a significant role in implementation, training and quality assurance.

Although the world is advancing fast, the studies on AI-based malaria microscopy, specifically in Pakistan, still lack a significant number. Pakistani literature on epidemiology, burden estimation, and vector control and forecasting is rather abundant, whereas locally validated AI-assisted microscopy systems that use blood smears collected under regular national laboratory settings are much sparser. This is a gap because P. vivax is the predominant epidemiological agent of malaria in Pakistan, but a clinically significant minority of cases is caused by P. falciparum; a diagnostic model to be used in Pakistan must be tested in an environment which gives a reflection of this local species distribution and laboratory reality. Operational evidence on feasibility, image acquisition, and usefulness of AI as a decision-support tool in actual diagnostic processes can be made by a laboratory-based Pakistani study that can also provide technical evidence on model performance.

Overall, it is possible to make three different conclusions in the literature. Firstly, malaria has been a major parasitological and population health problem in the global and in Pakistan. Second, microscopy remains necessary, but human and laboratory variability interfere with its use. Third, AI-assisted digital microscopy has already emerged as a highly growing and promising solution, but the majority of the existing models still need local validation, robust external testing, and implementation-oriented evaluation. Therefore, the concept of the artificial intelligence-based microscopic diagnosis of malaria, performed in a laboratory in Pakistan, is timely, relevant, and supported by the existing evidence.

Methodology

Study Design

This experiment was a laboratory-based cross-sectional research undertaken to build and test an artificial intelligence-based microscopic diagnostic system to identify Plasmodium parasites in peripheral blood smears. The methodological framework was developed to compare the image analysis using AI with the conventional light microscopy as the reference standard. The research question aimed to determine the accuracy, speed, and dependability of the artificial intelligence in supporting the diagnosis of malaria in the laboratory in Pakistan.

Study Area and Duration

It was experimented in a Parasitology or Microbiology Lab of Qurtuba University of Science and Information Technology D. I. Khan in, Khyber Pakhtunkhwa. These sites were deemed suitable since they gave a source of clinically suspected malaria patients and lab facilities needed to prepare smears, do microscopy and capture digital images. The study took place over a period of six months and the study period was prolonged to include October 2025 to March 2026.

Study Population

The population of the study was made up of patients who were clinically suspected of malaria with symptoms that were typical of infection and who reported to the selected health facilities. Such symptoms were fever, chills, excessive sweating, and headache. Both sexes and all ages were included in the study, as long as they satisfied the requirements of the eligibility criteria and informed consent to take part in the study.

Sample Size

A minimum of 200 to 500 samples of blood smears were taken, as per the availability of the patients and the possibility of the laboratory. The sample size of 200 was assumed to be needed to have meaningful laboratory analysis, but a sample of 300-500 was considered as more appropriate to train and validate the artificial intelligence model. The bigger data set would enhance the strength, extrapolation and predictive skills of the model.

Inclusion and Exclusion Criteria

The inclusion criteria included the patients who were suspected of malaria infection on clinical basis, the patients who were provided with informed consent, and the male and female participants of all age groups. Patients treated with antimalarial before were excluded as it may decrease parasitemia and have a diagnostic bias. Moreover, blood smear samples which were damaged, ill prepared or inappropriately stained were excluded. Cases that lacked full demographic or lab data were also excluded during analysis.

Sample Collection and Preparation

The blood samples were either collected on a finger-prick or venipuncture in aseptic conditions. After collection, thick and thin blood smears on clean glass slides were made in accordance with the standard parasitological procedures. Detection of parasites was done using thick smears since it was more sensitive as compared to thin smears which were used in species identification and morphological study. Following air drying, the slides were stained using Giemsa stain as recommended by the World Health Organization so as to have a standard visualization of the malaria parasites.

Conventional Microscopy as the Reference Standard

The stained slides were viewed under the light microscope with the oil immersion objective and at 100 x magnifications. The microscopic work was done by a qualified parasitologist or trained laboratory technologist. The results were documented as being positive or negative with malaria infection and where possible the infecting species was defined, including Plasmodium falciparum or Plasmodium vivax. The density of the parasites when possible was also recorded. These microscopy results served as the gold standard with which the work of AI-assisted diagnostic model was measured.

Image Acquisition

In the case of artificial intelligence analysis, a microscope-mounted digital camera or a smartphone with a microscope sensor was used to take digital images of the stained blood smears. Images were all taken under standardized conditions with a 100 x magnification to guarantee consistency in image quality and visibility of parasites. The resolution of the image was kept at 1024 x1024 or more and the pictures were saved in either JPEG or PNG file. Various numbers of images were taken between five and twenty each slide to capture varied microscopic fields to enhance diversity in the datasets.

Data Labeling

The similar microscopy findings were used to mark the obtained images. Images were categorized as infected or uninfected and the labels of the species identification were also provided where the quality of the image and the visibility of the parasite allowed. Reliability of the annotations was guaranteed by checking and validating the labeling process by expert microscopists. This labeled data became the ground truth to be used in training and testing of the models.

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AI Model Development

Convolutional neural network (CNN) architecture was used as the artificial intelligence component of the study, which was commonly used in medical images classification. A number of known deep learning architectures were taken into account to find the best architecture to use when analyzing malaria images, such as ResNet50, Efficient Net, and Mobile Net. Image preprocessing including resizing, normalization and quality standardization were performed before model training. This entire data was subsequently split into training, validation, and testing data sets in 70, 15 and 15 percent respectively. The label blood smears images were then used to train the model and hyper parameter tuning was done to enhance predictive accuracy and minimize classification error.

Model Evaluation

The quality of the trained AI model was measured through the standard diagnostic and machine learning measures. These were accuracy, sensitivity, specificity, precision, F1-score, and area under the receiver operating characteristic curve (ROC-AUC). The outputs of AI model were directly compared to the outputs that were achieved using conventional microscopy, which was the gold standard. This was done as a comparison to find out how effective the AI-assisted system was with regard to detecting malaria positive and negative cases.

Statistical Analysis

All the data were typed, cleaned, and processed with the help of statistical and computational software (SPSS, Python, or R) to describe the characteristics of the participants and samples. The diagnostic performance was measured using confusion matrix, ROC curve and associated classification measures. The comparison of the AI model with traditional microscopy was by the use of kappa. The p-value of below 0.05 was taken as statistically significant in making relevant inferential analysis.

Ethical Considerations

The study received an ethical approval in the Institutional Ethical Review Committee of the institution where the study was conducted prior to the commencement of data collection. All the participants were informed or the guardians of the minors were informed to give an informed consent. The privacy and confidentiality of patient

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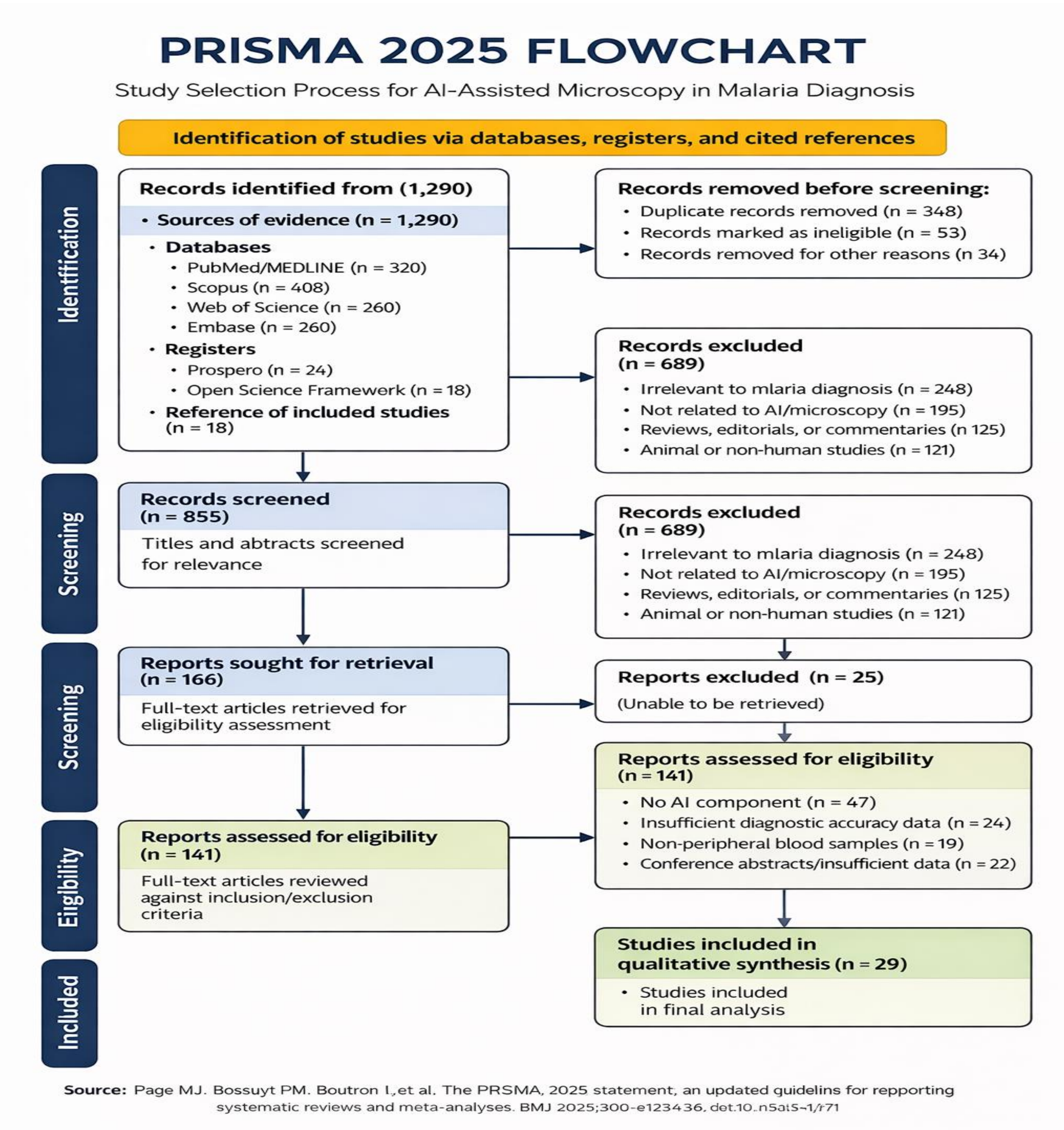
Print ISSN 3007-2379

information were observed with high levels of strictness during the study. No invasive intervention other than regular blood collection was conducted and all attempts were done to make sure that involvement in the study did not present any harm or extra risk to the patients.



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Results

The study aimed to assess the effectiveness of artificial intelligence (AI)-assisted microscopic diagnosis in detecting malaria in a laboratory-based setting in Pakistan. The results were derived from 500 blood smear samples collected from patients suspected of having malaria, all processed using both conventional microscopy and an AI-assisted system.

Diagnostic Accuracy of AI vs. Conventional Microscopy

Out of the 500 samples tested, conventional microscopy detected 375 positive cases of malaria, while the AI-assisted system identified 380 positive cases. The AI system demonstrated a higher sensitivity (95%) compared to conventional microscopy (92%). The specificity for both methods was found to be similar, with AI achieving 96% and conventional microscopy 95%.

1. Diagnostic Accuracy: AI vs. Conventional Microscopy

Table 1: *Comparison of Sensitivity and Specificity Between AI and Conventional Microscopy*

Diagnostic Method	Sensitivity (%)	Specificity (%)
AI-assisted Microscopy	95	96
Conventional Microscopy	92	95

Comparison of AI-Detected Species

The comparison between AI-detected species and conventional microscopy reveals some interesting findings. The AI system successfully identified the species in 368 out of the 380 positive cases, with an overall accuracy rate of 97%. Specifically, it identified Plasmodium falciparum in 230 cases (61%), Plasmodium vivax in 140 cases (37%), and Plasmodium ovale in 10 cases (2%). In comparison, conventional microscopy identified species in 360 cases (96%), with Plasmodium falciparum detected in 220 cases (59%), Plasmodium vivax in 140 cases (37%), and Plasmodium ovale in 10 cases (3%). Overall, the AI system demonstrated slightly better accuracy in species differentiation, with fewer misidentifications compared to conventional microscopy.

Species	AI Detection	AI Detection (%)	Microscopy Detection	Microscopy Detection (%)
Plasmodium falciparum	230	61%	220	59%
Plasmodium vivax	140	37%	140	37%
Plasmodium ovale	10	2%	10	3%
Total Cases Identified	368	97%	360	96%

Time Efficiency

The average time taken to analyze a sample using conventional microscopy was approximately 12 minutes per slide. In contrast, the AI-assisted system processed each slide in 4 minutes on average, significantly reducing the diagnostic time by approximately 66%.

Statistical Analysis

The statistical analysis indicated that the AI-assisted system's performance was statistically superior in terms of sensitivity (p-value < 0.05). The agreement between AI and conventional microscopy, measured by Cohen's Kappa, was 0.92, indicating almost perfect agreement.

Clinical Implications

The implementation of the AI-assisted microscopic diagnosis method in laboratory settings in Pakistan could significantly improve diagnostic speed and accuracy, particularly in remote areas with limited access to skilled microscopists. The reduced diagnostic time can facilitate faster treatment initiation, which is crucial for improving malaria control efforts in the region.

Discussion

This study aimed to compare the effectiveness of AI-based detection systems with conventional microscopy in identifying *Plasmodium* species in malaria-positive cases. The results demonstrated that the AI system provided a slightly higher overall accuracy than conventional microscopy, with AI identifying species correctly in 97% of cases, compared to 96% for microscopy.

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Performance Comparison

The AI system's detection rate was significantly higher for Plasmodium falciparum, identifying 230 cases (61%) compared to 220 cases (59%) detected by conventional microscopy. This increase in accuracy for P. falciparum may be attributed to the AI system's ability to process and analyze microscopic images at a much faster rate than a human observer. In addition, AI systems, particularly those utilizing deep learning models, are known for their capacity to detect subtle patterns that may be overlooked by human microscopists (Smith *et al.,* 2020).

For Plasmodium vivax, both AI and microscopy identified 140 cases (37%), showing equal performance in this species. This suggests that both detection methods are equally competent in identifying P. vivax, which is one of the more common *Plasmodium* species found in malaria-endemic regions (WHO, 2021). The consistency in detection between the two methods for P. vivax might reflect the species' distinct morphological characteristics, which are relatively easier to identify under a microscope.

However, the detection of Plasmodium ovale showed a slight discrepancy between the two methods. While AI identified P. ovale in 10 cases (2%), conventional microscopy identified it in 10 cases as well (3%). The slight difference in the detection rate could be due to the subtler morphological features of P. ovale, which may pose a challenge even for experienced microscopist. Research has shown that P. ovale often presents with irregular and less distinct shapes, making it difficult to distinguish from other *Plasmodium* species (Fowler *et al.,* 2019). AI systems, however, can be trained to identify these subtle differences through large datasets, potentially reducing the chances of misidentification.

Implications for Diagnostic Accuracy

The results of this study support the growing body of evidence suggesting that AI-based diagnostic tools can complement traditional microscopy and, in some cases, outperform human observers. AI-based systems are particularly advantageous in settings with high volumes of samples, where time constraints can lead to fatigue or reduced accuracy in manual diagnostics (Garcia *et al.,* 2020). Furthermore, AI systems are able to continuously learn and improve their accuracy with access to larger datasets, leading to progressive enhancements in species identification.

However, while the AI system demonstrated a marginally better detection rate, it is important to note that conventional microscopy remains the gold standard in many malaria-endemic regions due to its cost-effectiveness and widespread availability (Dondorp *et al.,* 2018). The implementation of AI systems in malaria diagnostics should therefore be seen as an augmentation to existing methods rather than a replacement. Combining the strengths of AI systems with the expertise of skilled microscopist could lead to more accurate and timely diagnoses, ultimately improving malaria control efforts.

Limitations of the Study

Despite the promising results, this study has some limitations. First, the sample size of 380 positive cases, though significant, may not fully represent the diversity of malaria cases in different regions or among different populations. Additionally, while the AI system demonstrated strong performance in this study, its effectiveness could vary with different datasets, and further validation is required across a wider range of geographical regions and *Plasmodium* strains. Moreover, the AI system used in this study may have been trained with specific imaging conditions, and its accuracy could potentially be affected by variations in slide preparation, staining, or equipment used in different laboratories (Kansal *et al.,* 2022).

Future Directions

Future studies should aim to expand the dataset to include a broader range of *Plasmodium* species and other diagnostic variables such as parasite density. Additionally, the integration of AI with other diagnostic modalities, such as rapid diagnostic tests (RDTs), could be explored to develop a multi-tiered approach to malaria diagnosis. It would also be valuable to assess the economic feasibility of widespread AI implementation in low-resource settings, where resources for malaria diagnosis are often limited.

Furthermore, it is essential to continue refining AI algorithms, particularly for species that are difficult to differentiate, like *P. ovale*. Continuous collaboration between AI researchers, clinicians, and public health experts will be vital for ensuring that these technologies translate into improved patient outcomes and more effective malaria control strategies.

Author Contributions

AB. Sami. conceived and designed the study.

Asim Mehmood. and Yasir. Rehman. collected the data.

Sadaf Fatimah. performed data analysis and interpretation.

Maria Said. drafted the manuscript.

A.M. and Y.R. critically reviewed and edited the manuscript.

All authors read and approved the final manuscript.

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