

COMPARATIVE STUDY BETWEEN PCR, ICT AND MICROSCOPY IN DIAGNOSE OF *PLASMODIUM FALCIPARUM*

Hana Ibraheem and Elamin Abdelkarim Alamin*

Department of Medical Parasitology, Faculty of Medical Laboratory Sciences, Al Neelain University, Khartoum, Sudan.

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ABSTRACT

Microscopic detection of *Plasmodium* species has been the reference standard for the diagnosis of malaria for more than a century. However, maintaining a sufficient level of expertise in microscopic diagnosis can be challenging, particularly in non-endemic countries. The main objective of this study was to compare the performance of nested PCR with expert microscopy and rapid test as a means of detecting *Plasmodium falciparum* parasites in different hospitals of Khartoum state. Thirty venous blood samples was collected in to a dry EDTA container and preserved at refrigerator for subsequent processes. Direct microscopy was carried out by preparing thin and thick blood films and staining with Giemsa's stain, and then ICT was carried out. DNA was extracted from samples using guanidine hydrochloride extraction method to detect *P.falciparum* DNA. With comparison PCR as gold standard method, there was high sensitivity and specificity detected by microscopy 50%, 54% respectively. In the SD® ICT assay there was sensitivity (25.5%) and low specificity (56%). ICT diagnostic technique cannot be recommended as a sensitive and specific tool for malaria diagnosis. PCR appears to be a useful method for detecting *Plasmodium* parasites.

INTRODUCTION

Microscopic detection of parasites on Giemsa stained blood smears has been the reference standard for malaria diagnosis in laboratories for more than a century, it is an imperfect standard highly dependent on the technical expertise of the microscopists. Despite an excellent health care system with specific and effective therapy options, fatalities do occur in so-called developed countries due to gaps in patient's and physician's knowledge. The ability to maintain the required level of expertise in malaria diagnostics may be problematic especially in peripheral medical centers in countries where the disease is not endemic [1]. The World Health Organization has recognized the “urgent need for simple and cost-effective diagnostic tests for malaria to overcome the deficiencies of

[both] light microscopy” and clinical diagnosis [2]. Consequently, recent efforts have focused on developing sensitive and specific non-microscopic malaria diagnostic devices including those based on the detection of malaria antigen in whole blood [3,4]. The objective of this study was to examine the performance of the SD® ICT test compared with a blinded polymerase chain reaction (PCR) and expert microscopic analysis for the diagnosis of human plasmodium falciparum parasite in symptomatic patients.

MATERIALS AND METHODS

Microscopy

A need for reliable laboratory diagnostic microscopy services is critical; Giemsa stained slide microscopy is regarded as the most suitable diagnostic instrument for malaria control because it is inexpensive to perform, able to differentiate malaria species, and quantify of parasites.

Corresponding Author

Elamin Abdelkarim Alamin

Email: - elaminpara72@yahoo.com

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Rapid Diagnostic Test (RDT)

Rapid diagnostic test is a device that detects malaria antigen in a small amount of blood, usually 5–15 μ L, by immunochromatographic assay with monoclonal antibodies directed against the target parasite antigen and impregnated on a test strip. The result, usually a colored test line, is obtained in 5–20 min. RDTs require no capital investment or electricity, are simple to perform, and are easy to interpret. First-generation of rapid diagnostic products relied on the detection of the histidine-rich protein II (HRP II) antigen of *P. falciparum* and therefore could not detect other *Plasmodium* species. A newer generation of rapid diagnostic devices based on antigen capture with immune-chromatographic (ICT) strip technology and use of monoclonal antibodies to HRP II for the detection of *P. falciparum* as well as aldolase, a pan-*Plasmodium* antigen, thus facilitating identification of non-falciparum infections. The test results were independently examined and interpreted by three observers blinded to the microscopic and PCR results. The final results of the test were recorded as either negative or positive based on the majority agreement. The readers also graded the assays results (As band intensity for the HRP II and *pan-Plasmodium* antigen bands) ranging from 0 (negative: no visible reaction for either HRP II or pan-malaria antigen) to 4+ (strongly positive reaction for antigen) [5,6].

Polymerase chain reaction

Detection and malaria species identification by PCR were performed as follow [5,6]: genomic DNA was extracted from whole blood samples using guanidine hydrochloride following the manufacturer's instructions. A 5 μ L aliquot of the DNA extract was used in a nested PCR assay to amplify a segment of the *Plasmodium* 18S ribosomal RNA gene. The resulting PCR product was analyzed by electrophoresis on a 1% agarose gel stained with ethidium bromide [7].

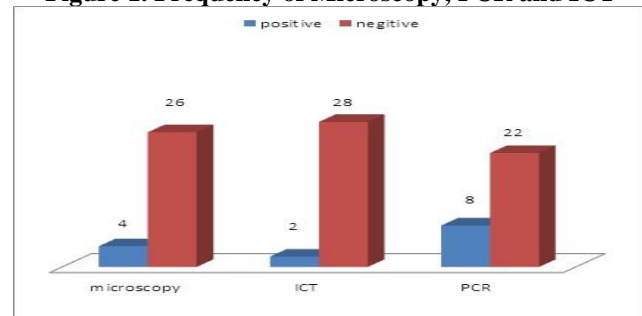
Data analysis

Data was computerized by using soft program statistical package for social science (SPSS) and manually using master sheet.

RESULTS

During the study period a thirty venous blood samples diagnosed by different types of methods (ICT result: 6.7% positive and 93.3% negative. PCR result: 26.7% positive and 73.3% negative, and the microscopy result 13.3% positive and 87% negative.) All details are shown in figure 1.

Figure 1. Frequency of Microscopy, PCR and ICT



When compared with the PCR, the sensitivity of SD® ICT assay was 25.5% for the detection of pure *P. falciparum* infections, and specificity was 56%. The corresponding of predictive value for SD®ICT and PCR was significant (0.015); the results of the SD® ICT test compared with PCR-based diagnosis are shown in Table 1.

Results of the SD ® ICT test compared with microscopic diagnosis are shown in Table 2.

Table 3 shown the result of microscopy compared with PCR. The sensitivity and specificity were 50%, 54% respectively, and p value was significant (0.00).

Table 1. Comparison between PCR & ICT

	PCR	ICT	P value
Positive	8	2	0.015
Negative	22	28	
Total	30	30	

Table 2. Comparison between microscopy & ICT

	Microscopy	ICT	P value
Positive	4	2	0.00
Negative	26	28	
Total	30	30	

Table 3. comparison between microscopy and PCR

	Microscopy	PCR	P value
Positive	4	8	0.00
Negative	26	22	
Total	30	30	



DISCUSSION

Microscopy is regarded as the most suitable diagnostic instrument for malaria control because it is inexpensive to perform, able to differentiate malaria species, and quantify of parasites. As microscopy depends on the good microscopists there may be false negative and positive results.

In spite of that PCR was both sensitive and specific for the diagnosis of malaria; there was false negative result occur this can be attributed to limitations that can affect the accuracy of the method. Such as selection of appropriate primers, methods used for collection, storage of blood samples and extraction methods can all affect PCR performance.

This in agreement with Jelinek *et.al* [8] who reported that the sensitivity of PCR was as much linked to parasite density. They found that sensitivity of PCR was affected by both parasite density and by geographic differences in parasite populations.

The ICT method was faster than microscopy and PCR and applicable when microscopic examination is impossible due to autolysis, but also there was some factors can give

false negative results due to: bad storage and impairment to detect the sexual stages (HRP2 of SD® ICT is anti merozoite). False positive result occurs: if there is rheumatoid factor positive patients, or due to existent of HRP2 about two weeks after complete clearance of *P. falciparum* parasite from blood circulation by treatment [9,10].

CONCLUSION

During this study ICT diagnostic cannot be recommended as a sensitive and specific tool for malaria diagnosis. PCR appears to be a useful method for detecting *Plasmodium* parasites during active malaria surveillance. Although PCR is more expensive and time consuming but may be used as confirmatory test in highly endemic areas where asymptomatic parasitaemia is common.

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