Rapid Detection of Malaria Parasite by Toluidine Blue Method: A New Staining Method

Vamseedhar Annam, Chakkirala Nalini Mohan, Vazhayil Ramunny Mrinalini

Department of Pathology, Melmaruvathur Adhiparasakti Institute of Medical Sciences and Research, Melmaruvathur, Tamil Nadu, India

ABSTRACT

Background: Malaria is a commonest mosquito-borne infectious disease worldwide. Early identification and management of malaria prevents complications and mortality. Identification of the malaria mainly relies on detection of the parasite on blood smears. The present study was conducted to compare Toluidine blue method with Leishman method for detection of malaria parasite and also to study the efficacy and advantages of using Toluidine blue method. **Materials and Methods:** In 540 consecutive patients with clinical suspicion of malaria, peripheral smears were prepared. Smears were processed for both conventional Leishman method and Toluidine blue method simultaneously. The significance of Toluidine blue method over Leishman method was analyzed using Chi-square (χ^2) test. **Results:** Out of 540 smears, 28.3% (153/540) were positive for malaria parasite on conventional Leishman method, while the smear positivity was more by Toluidine blue method to 33.3% (180/540) [*P* value < 0.01]. The remaining 66.67% (360/540) were negative by both Toluidine blue method and conventional Leishman method. **Conclusion:** The Toluidine blue method is simple, rapid, inexpensive, and easily available. The implementation of Toluidine blue method clearly improves microscopic detection of malaria parasite and can be a useful contribution to routine hematology even at rural health sectors.

Key words: Leishman method, Malaria, Peripheral smear, Toluidine blue method

INTRODUCTION

Malaria is a tropical disease causing 1.5-2.7 million deaths yearly worldwide.[1] The definitive diagnosis depends on the demonstration of malaria in blood smears. The Jaswant Singh and Bhattacharji stain requires expertise to achieve good color contrast.[2] In addition, the Fields stain has low sensitivity possibly due to undefined ring stage,[3] whereas Giemsa stain is labor-intensive, time-consuming, and difficult to detect low levels of parasitemias.[4,5] The Acridine orange fluorescent method requires fluorescence microscope, which is expensive.[6] Thus, there is requirement of newer method that is rapid and cost-effective. Hence, the present preliminary study was conducted.

MATERIALS AND METHODS

The blood samples were collected under aseptic precautions from 549 patients with clinical suspicion of malaria during febrile attacks. The patients were referred from both inpatient and outpatient departments of Sree Siddhartha Medical College, Hospital and Research Center, Amruth Biological and Clinical Services Pvt. Ltd and Melmaruvathur Adhiparasakti Institute of Medical Sciences and Research under Tumkur district, Bangalore district and Kancheepuram district, respectively, over a period of 4 years. Exclusion criteria were treatment for malaria or initiation of malaria treatment before sampling was performed. Two smears were prepared from each blood sample and were processed for conventional Leishman method and Toluidine blue method.

The following Toluidine staining procedure was implemented:

1. The air-dried blood smears were stained with Toluidine Blue stain for 1 minute.
2. The slide was rinsed with deiodinised water for 1 minute, air-dried and examined under high power (×, 400), which was confirmed under oil-immersion (×, 1000).

The malaria parasite appears as deep violet color containing golden brown pigment [Figure 1, ×1000, Gametocyte

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Address for correspondence:
Dr. Vamseedhar Annam, E-mail: vamseedhar_a @ yahoo.com

of *Plasmodium vivax*], [Figure 2, ×1000, Gametocyte of *Plasmodium falciparum*], [Figure 3, ×1000, Ring forms of *Plasmodium vivax*] under light microscopy and the background appears pale green to colorless.

The data were processed using test of association (Chi-square test).

RESULTS

A total of 549 blood samples were collected from patients with clinical suspicion of malaria. Of these, 540 samples were evaluated and the remaining 9 samples were eliminated because of poor quality of smears. The age ranged from 3 to 81 years. Male: Female ratio was 3:1. Among the malaria parasite positive smears, *Plasmodium vivax* was the most common with 60% (108/180) and *Plasmodium falciparum* with 40% (72/180).

The degree of the Kappa agreement between both the tests, which was near perfect 0.88 (Landis and Koch).

As showed in Table 1, by routine Leishman method, 28.3% (153/540) were positive for malaria parasite, while the smear positivity increased to 33.3% (180/540) on Toluidine blue method. The remaining 66.67% (360/540) were negative by both Toluidine blue method and routine Leishman method.

DISCUSSION

Malaria continues to be one of the major health problems in the developing countries, accounting for significant morbidity and mortality. For the increasing incidence of malaria, rapid detection and effective treatment of malaria is a prerequisite to reduce the chances of transmission. This has given an impetus for development of simple and rapid methods for the detection of malaria. Identification of the malaria parasite on peripheral smear examination by light microscopy still remains the gold standard in malaria endemic countries.[7] Light microscopy has the advantages that it is sensitive, informative, relatively inexpensive, provides permanent record, and can be shared with other disease control programs.

Table 1: Comparison of leishman method with toluidine blue method for the detection of malarial parasite

Toluidine method	Leishman method		Total
	Positive	Negative	
Positive	153	27	180
Negative	00	360	360
Total	153	387	540

$\chi 2=422.80$, df=1, *P* value=<0.01

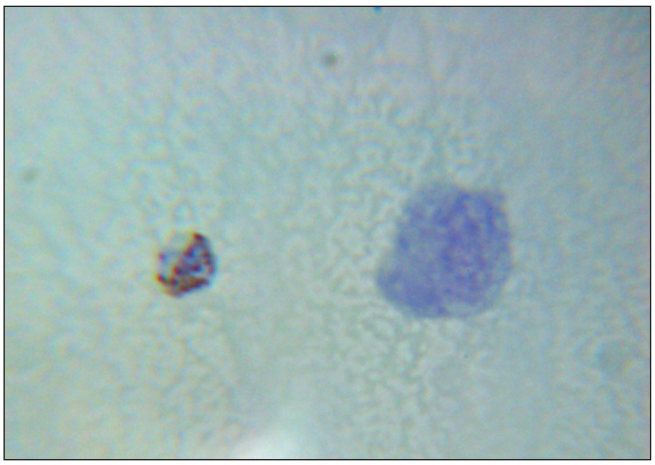

Figure 1: [Toluidine blue stain, ×1000]: Gametocyte of *Plasmodium vivax* are seen

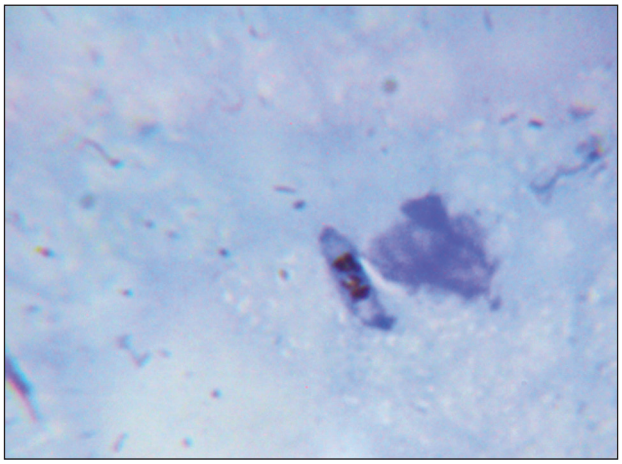

Figure 2: [Toluidine blue stain, ×1000]: Gametocyte of *Plasmodium falciparum* are seen

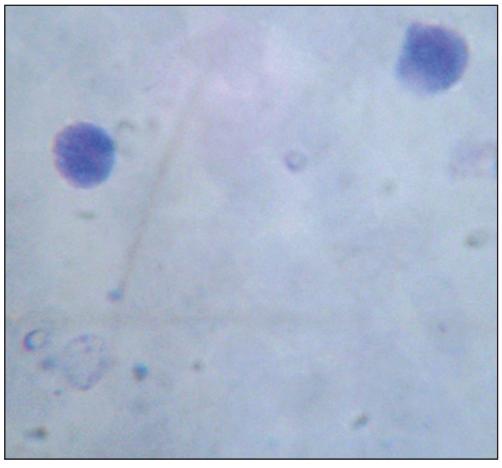

Figure 3: [Toluidine blue stain, ×1000]: Ring forms of *Plasmodium vivax* are seen

In the present study, a new staining method was performed by using Toluidine blue stain and compared with the routinely practiced Leishman stain method. Toluidine blue method correlated well with traditional Leishman method. There are no previous literatures regarding the use of Toluidine blue method for the detection of malaria parasite.

Toluidine blue method has the advantage that screening is much faster. The erythrocytes in the peripheral smear appears pale green possibly due to binding of the stain only to glycocalyx of the erythrocyte membrane and thus exhibiting metachromasia. The leucocytes appear purple in color, while parasites including ring forms can be easily identified by the deep violet parasite containing golden brown pigment against a relatively pale green to clear background. If any doubt remains, it is possible to mark the individual suspicious areas with diamond objective marker on the slide, then restained over the Toluidine blue stain by Leishman method and examine under oil-immersion lens. The smear positivity for Toluidine blue method increased to 33.3% as compared with Leishman method (28.3%). The possible reason could be easy identification of the ring forms by Toluidine blue method, which were missed or misinterpreted for platelets by Leishman method.

CONCLUSION

We conclude that Toluidine blue method could be a rapid alternative method to conventional Leishman method for detection of malaria parasite. The advantage of Toluidine blue method is that Toluidine blue stained slides can be examined under high magnification allowing for much larger areas of the smear to be examined in a short period of time. The use of Toluidine blue method greatly improves the diagnostic value especially in patients with low density of parasites and ring forms that are likely to be missed by Leishman method. Thus implementation of Toluidine blue method clearly improves microscopic detection and can be a useful contribution for screening of malaria parasite in routine hematology. This would be of benefit to the patients to receive an early and effective treatment. In addition, Toluidine blue method is simple, easy, cost-effective, and rapid. Hence, Toluidine blue method can be considered for malaria control programs.

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