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Original Research Article

Novel Technique for Rapid Screening of Malarial Parasite by Toluidine Blue Staining

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Abstract

Introduction: Malaria is a tropical disease causing millions of death worldwide. Early detection and management prevents complications and mortality. Definitive diagnosis depends on demonstration of malaria in blood smears. *Aims & objectives:* In this study we intend to evaluate Toluidine blue utility and efficacy in screening & species identification of malaria parasite in peripheral smear. *Materials and methods:* In 134 patients with clinical suspicion of malaria, peripheral smears were prepared and were processed with both conventional Leishman method and Toluidine blue method simultaneously. *Results:* Out of 134 smears, 51 were positive for malaria with male preponderance and age incidence being more between 41-50 yrs. Predominant species was Plasmodium vivax (76.47%). Leishman stain showed decreased sensitivity (84.3%) and negative predictive value (91.2%) when compared to Toluidine blue stain. *Conclusion:* Toluidine blue staining method helps to identify parasites even at low power objective allowing screening more areas in shorter time. It is specifically useful in low parasite index cases and in species identification

Keywords: Malaria Parasite; Leishman Stain; Toluidine Blue; Peripheral Smear.

Introduction

Malaria is mosquito borne infectious disease accounting for 429,000 deaths [1]. It is transmitted by infected female anopheles mosquito. Africa is the country having highest morbidity & mortality due to this disease because of certain factors like favourable weather for transmission, increased vector population & increased incidence of plasmodium falciparum infection causing high mortality. India also has high malaria burden. To prevent the morbidity and mortality due to malaria, early diagnosis is essential. Various stains like Giemsa stain, Jaswant Singh Bhattacharya stain, Fields stains and Acridine orange fluorescent method [2] are available for detecting malaria parasite. In our study we tried to evaluate the

efficiency and utility of Toluidine blue stain in comparison with conventional Leishman stain for detecting malaria parasite in peripheral smear.

Methods

This study was conducted in Narayana Medical College, Nellore, India for a period of one year. Blood sample were collected from 134 patients who attended the hospital with fever and were suspected to have malaria. The patients already receiving the treatment for malaria were excluded in our study. Two peripheral smears were prepared from the blood sample collected in EDTA vacutainer. One slide was stained by Leishman stain and the other was stained by Toluidine blue stain. To prepare Leishman stain, 1.5gms of Leishman powder was mixed with 500ml of methanol. The mixture was kept at 37°C with occasional shaking for overnight. Leishman stain was poured on the smear and left for 2 min. Afterwards double quantity of distilled water was added. This was thoroughly mixed and kept for 8 minutes followed by washing with tap water. Backside of the slide was wiped and kept for drying [3]. To prepare Toluidine blue stain, 5gms of Toluidine blue stain was mixed with 400ml of distilled water & 100ml of 95% alcohol. This mixture was kept for overnight at room temperature, then the stain is filtered & used. On the air dried smear pour the Toluidine blue stain & wait for 1 minute. Then wash the smear in deionised water. Backside of the slide is clearly wiped and allowed to dry for 5 minutes. Leishman stained smear and Toluidine blue stained smear are examined under the microscope. Findings were noted and analysed.

Results

Our study included 134 patient who were suspected to have malaria infection. Out of 134 patients, 51 patients were diagnosed to have malarial infection. Maximum number of patients having malarial infection was noted in the age group of 41-50 years (Table 1). Slight male predominance was noted with male to female ratio of (1:6:1)(Table 2).

Out of 51 cases of malaria predominant cases were of Plasmodium vivax (76.47%) when compared to Plasmodium falciparum (23.53%) (Table 3). Out of 51 malaria cases diagnosed by Touidine blue stain, only in 43 cases (84.3%) malarial parasite was identified in Leishman stained smear. Remaining 8 cases (15.7%) were diagnosed as negative for malaria (Table 4). Leishman stain showed decreased sensitivity (84.3%) and negative predictive value (91.2%) when compared to Toluidine blue stain (Table 5).

Discussion

Malaria accounts for significant morbidity & mortality in the developing countries. Early detection of malaria and effective treatment can reduce the chance of complications and transmission of malaria. Identifying malaria parasite by light microscope in peripheral smear is the gold standard for diagnosing the condition in the malaria endemic countries [4].

Thick and thin smears are used for detecting malarial parasites. For the preparation of thick

Table 1: Age wise distribution among malarial patients

Age in years	Total cases		Malaria positive cases	
	Number of cases	Percentage	Number of cases	Percentage
0 -10	15	11.2	1	1.96
11 – 20	40	29.8	13	25.5
21- 30	25	18.6	9	17.64
31 – 40	23	17.3	7	13.72
41 – 50	18	13.4	18	35.3
51 – 60	8	5.9	2	3.92
61 – 70	5	3.8	1	1.96

Table 2: Sex wise distribution of malaria patients

Number of malaria [patients	Male	Female
51	32 (62.75%)	19 (37.25%)

Table 3: Distribution of Plasmodium species

Number of cases	Plasmodium vivax	Plasmodium falciparum
51	39 (76.47%)	12 (23.53%)

Table 4: Malarial parasite detection by Leishman and Toluidine blue stain

Toluidine blue stain	Leishman stain		Total number of cases
	Positive	Negative	
Positive	43 (84.3%)	8 (15.7%)	51
Negative	-	83	83

Table 5: Comparision between Leishman and Toluidine blue stain

	Leishman stain	**Toluidine stain**
Sensitivity	84.3%	100%
Specificity	100%	100%
Positive predictive value	100%	100%
Negative predictive value	91.2%	100%

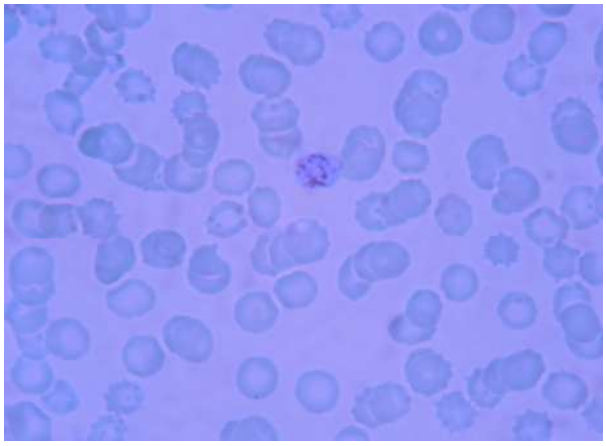

Fig. 1: Schizont of Plasmodium vivax (Toluidine blue, X1000)

smear 20 µl of whole blood is needed which can be capillary or anticoagulated venous blood. Drop of blood is placed in centre of the slide and 3 to 6 circular strokes are given to spread the drop by the corner of the another slide. Thickness should be such that through the blood film normal print can be seen but letters cannot be read. Leave the slide to dry for 20 minutes. After drying dehaemoglonization should be done by using Glacial acetic acid for few seconds. Then stain the slides by using Giemsa solution (3.5%). Staining requires 20 minutes of time and preparation of Giemsa solution requires technical expertisation [5].

This technique is sensitive in detection of malaria parasite but not suitable for malaria parasite species identification as there is swelling of parasite & distortion of morphology of parasite.

Other methods for detection of malaria parasite which are expensive are Quantitative buffy coat method where ultraviolet illuminated tubes are used to observe the parasitized red blood cells. Acridine orange fluorescent microscopy can also be used for malaria parasite detection but it is also expensive and need expertise [6]. Malaria antigen detection is another diagnostic test which can rapidly detect malarial infection but is expensive than microscopy.

William Henry Perkin discovered Toluidine blue stain in 1856. Since than it has been used to stain mast cell granules, connective tissue mucins, malignant oral cells, micro organising like Helicobacter, Corynaebacterium diphtheria and Mycobacterium [7]. This stain can also be used to evaluate malarial parasite in peripheral smear. Leishman stain and Giemsa stain requires maintenance of the optimal pH of 7.2 where as Toluidine blue stain does not require maintenance of pH and hence can be used in the field setting. Toluidine blue stain can be used to detect the cases, where there is low parasite index and smear can be screened in shorter time as only the parasites stain differently. Studies done Annam V et al. (2013) and Shravan N et al. (2010) have shown that Toluidine blue stain has higher detection rate, has more accuracy & more specificity when compared to Leishman stain [8, 9]. This coincided with our study which also showed high sensitivity & specificity for Toluidine blue stain.

Conclusion

Toluidine blue stain is simple and less expensive method which requires minimal expertise for preparation of smear. It contributes for the rapid screening of smear and helps in improving microscopic detection of malarial parasite. It has more efficacy than Leishman stain in detecting the parasite where there is low density and also in species identification.

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References

1. World Health Organization. World malaria report Geneva. 2016.pp.1-186.
2. Hemwani N, Chitnis DS, Dixit DS, Asolkar MV. Acridine orange stained blood wet mounts for fluorescent detection of malaria. India J Pathol Microbiology 1999;43:125-8.
3. Rupali Awale, Ratna prabha Maji, parag Patil, Raghavendra Lingiah, Ashok Kumar Mukhopadhyay, Subhadra slama, Toulidine blue: rapid & simple malaria parasite screening & species identification. Pan Afr Med J. 2017;28:27.
4. Warhurst DC, Williams JE. Laboratory diagnosis of malaria. J Clin Pthol 1996;49:533-8.
5. Bejon P, Andrews L, Hunt –Cooke A, Sanderson F, Gilbert SC, Hill AVS. Thick blood film examination for plasmodium falciparum malaria has reduced sensitivity and underestimates parasite density. malar J. 2006;5:104.
6. Wongsrichanalai. C, Pornisilapatip J, Namsiripongpun V, Webster HK, Luccini A, Pansamdang P et al. Acridine orange fluorescent microscopy and the detection of malaria in populations with low density parasitemia. Am J Trop Med Hyg. 1991;44(1):17-20.
7. JV. Sridhara G, Shankar AA, Toluidine blue: a review of its chemistry and clinical utility. J. Oral maxillofac Pathol 2012;16(2):251-55.
8. Annam V, MohanCN, Vazhayi lRM. Rapid detection of malaria parasite by toulidine blue method: a new staining method J Glob Infect Dis. 2013;5(4):153-55.
9. Shravan N, Yelikar BR. A novel use of Toluidine blue in diagnosing malaria. Australasian Med J. 2010;3(8):554.