Flagging Of Malaria Parasite In Hematology Analyzer With Its Correlation On Peripheral Smear And Rapid Diagnostic KIT.

Ayesha Ayyub[1], Ashutosh kumar[2], S.Dutta[3], Himanshu Joshi[4]

[1]Junior resident, Dept. of pathology, Teerthanker Mahaveer medical college & Research Centre, Moradabad.
[2]Associate Professor, Dept. of pathology, Teerthanker Mahaveer medical college & Research Centre, Moradabad.
[3]Professor, Dept. of pathology, Teerthanker Mahaveer medical college & Research Centre, Moradabad.
[4]Assistant Professor, Dept. of pathology, Teerthanker Mahaveer medical college & Research Centre, Moradabad.

ABSTRACT

Background: Malaria has long been known to human populations from across the world. Malaria remains the most important parasitic disease worldwide. Malaria is a potential medical emergency and should be treated accordingly. Routinely malaria is diagnosed using a combination of clinical observations, case history, and diagnostic tests, principally microscopic examination of stained slides. The objective of this study is to detect the flagging of malaria parasite in sysmex with correlation in peripheral smear and kit so that early detection of Malaria can be done with the most accurate result in lesser duration and cost. **Objectives**:-To detect the flagging of malaria parasite in hematology analyser & to correlate the flagging findings on peripheral smear and rapid diagnostic kit. **Methods:** the study was conducted in Department of Pathology in collaboration, Teerthankar Mahaveer Medical College and Research Centre (TMMC&RC), Moradabad, Uttar Pradesh. TMMC&RC .A total of 11o cases were enrolled with Patients presenting with fever suggestive of clinical features of malaria (high grade fever with chills) and found serologically positive by blood smear, Sysmex flagging or RDT. **Results:** On the basis of observations made in the present study, Sysmex flagging was positive in 92 (83.6%) cases, 94 (85.5%) were positive by RDT& PBS positivity was seen in 99 (90%) cases. Among 99 positive cases, 85 were positive for P. vivax, 9 were positive for P. falciparum and 6 were positive for mixed infection. As compared to RDT, Sysmex flagging had 90.4% sensitivity; 56.3% specificity; 92.4% positive predictive value; 50.0% negative predictive value and 84.5% accuracy for detection of malarial parasite. Whereas when compared to PBS, Sysmex flagging had 90.9% sensitivity; 81.8% specificity; 97.8% positive predictive value; 50.0% negative predictive value and 90% accuracy for detection of malarial parasite. Sysmex flagging had moderate and significant agreement with both RDT and PBS assessments. Flagging had a detection rate of 66.7%, 94.1% and 80% respectively for P. falciparum, P. vivax and mixed infections. **Conclusion:** The findings of present study showed that Sysmex flagging is a cost-effective highly efficacious tool for screening of malaria parasite and it showed a high level of agreement with conventionally used laboratory tests as well as peripheral blood smear.

Keywords: Malaria, Sysmex, Peripheral smear, RDT.

INTRODUCTION

Malaria has long been known to human populations from across the world. Malaria remains the most important parasitic disease worldwide. Malaria is the fifth cause of death from infectious diseases worldwide (after respiratory infections, HIV/AIDS, diarrheal diseases, and tuberculosis) and the second in Africa, after HIV/AIDS.[1]

Malaria is caused by infection with protozoan parasite belonging to the genus Plasmodium, transmitted by female Anopheles species mosquitoes. The most serious and sometimes fatal type of malaria is caused by Plasmodium falciparum. The other human malaria species, P. vivax, P. ovale, P. malariae, and sometimes P. knowlesi can cause acute, severe illness but mortality rates are low. In the year 2015, an estimated 212 million cases of malaria occurred worldwide (UI: 148–304 million).[2]

Malaria is a potential medical emergency and should be treated accordingly. Malaria causes significant morbidity and mortality worldwide.In developing nations, scarce resources lead to inadequate diagnostic procedures.

Routinely malaria is diagnosed using a combination of clinical observations, case history, and diagnostic tests, principally microscopic examination of stained

Name & Address of Corresponding Author
Dr. Ayesha Ayyub
Junior resident,
Dept. of pathology,
Teerthanker Mahaveer medical college & Research Centre, Moradabad.

slides. Clinical diagnosis offers the advantages of ease, speed, and low cost. But it can lead to over-diagnosis and contributes to misuse of antimalarial drugs therefore WHO has recommended that all clinically suspected malaria cases should be investigated immediately by microscopy and/or Rapid Diagnostic Test (RDT).[3]

In the limited resource settings, rapid detection tests are more practical at the point of care in communities where community health workers (CHWs) can be trained in their use, as they do not require electricity or special equipment. RDTs may also detect Plasmodium infection even when the parasites are sequestered in the deep vascular compartments and thus undetectable by microscopic examination of a peripheral blood smear.[4]

Advantage MAL Card/ErbaQik Malaria was also one of them and was approved by the WHO as a Rapid Diagnostic test.. This is a an antibody test that employs an immunoassay based on the 'sandwich' principle. The conjugate contains colloidal gold conjugated to monoclonal anti-pan specific pLDH(parasite lactate dehydrogenase)antibody. The test uses monoclonal anti-Pf pLDH antibody and monoclonal anti-PAN specific pLDH antibody immobilized on a nitrocellulose strip. Addition of test sample immediately followed with addition of buffer lyses the red cells for release of malarial parasite and thus the test provides immediate and rapid outcome.[5]

Although, light microscopy still remains the gold standard for detection of malarial parasite yet it has its own limitations. In an attempt to enhance the detection of malaria parasites in blood films, alternative methods have been introduced. Automated analyzers based on flow cytometry have evolved as an effective adjuvant diagnostic tool in malaria diagnosis. Despite not being specifically designed for detection of malaria related abnormalities, they have sensitivity for detection of malaria parasite, that comply with WHO malaria diagnostic guidelines, i.e., 95% in samples with >100 parasites/µl.[6]

The advantage of using flow cytometry based hematology analyzers for malaria balances the inter-observer variability in malarial parasite assessment using light microscopy. Complete blood count (CBC) by automated analysers is a routine diagnostic test that is almost always prescribed by the clinicians for any febrile patient. The automated analyzers have incorporated various methods for characterization of blood cell populations and provide accurate CBC.

Sysmex-XS 800i is an advanced automatic flow cytometry based system that performs performs analysis of WBC, DLC and parasite with an optical detector block based on the flow cytometry method using an semi-conductor laser. Detection of parasite is based on scattered light and fluorescence intensity. The hemozin pigment is ingested by leucocyte after rupture of Plasmodium schizont. The system in turn thus helps in flagging of malarial parasite and thus has the capability to limit the screening universe substantially. However, clinical usefulness of this system is yet to be established completely.[7]

Thus, these two methods have emerged as useful alternatives to provide a rapid and probably accurate diagnosis of Malaria parasite especially in a resource limited scenario. The staged use of flagging using flowcytometry method followed by rapid diagnostic test could not only save the resources but can help in obtaining more accurate results. Incidentally, both the methods are newly introduced and lack adequate clinical studies, hence the present study was planned to evaluate the usefulness of Sysmax XS 800i flow cytometry based automatic analyzer for flagging and to correlate its findings with rapid diagnostic kit and peripheral blood smear.

MATERIALS AND METHODS

Study Area: Department of Pathology in collaboration, Teerthanker Mahaveer Medical College and Research Centre (TMMC&RC), Moradabad, Uttar Pradesh. TMMC&RC is a tertiary care facility catering to patients from and around Moradabad district. The facility has state of the art-infrastructure and multi-specialty facilities and caters to a diverse demography of patients.

Study Population:-Patients presenting with fever suggestive of clinical features of malaria (high grade fever with chills) presenting to Outpatient Department and different wards of TMMC&RC.

No. of cases:- 110 cases

Inclusion Criteria:
1. All patients proved positive for malaria peripheral smear or by rapid optimal test or having a proven family history of malaria during the last one month.

Exclusion Criteria
1. Patients unwilling to participate in the study.
2. Not completing all the investigations.

Method of collection of data
1. Thin and thick blood smears by Leishman stain or Giemsa stain examination to detect the presence and type of malaria.
2. WBC ,RBC , platelet and parasite flagging obtained by automated counter.(SYSMEX XS 800 i)
3. Rapid diagnostic kit

RESULTS

The present study was carried out to evaluate the usefulness of flagging of malaria parasite in hematology analyzer. For this purpose, a total of 110 suspected cases of malaria (being Sysmex positive, RDT or smear positive) were enrolled in the study. Table 1 shows the age profile of patients enrolled in the study:

Table 1: Age Profile of Patients enrolled in the study.

SN	Age Group	No. of cases	Percentage
1.	<10 Years	14	12.7
2.	11-20 Years	27	24.5
3.	21-30 Years	23	20.9
4.	31-40 Years	16	14.5
5.	41-50 Years	15	13.6
6.	51-60 Years	11	10.0
7.	61-70 Years	4	3.6

Majority of patients were males (63.6%). There were 40 (36.4%) females. Male to female ratio was 1.75.All the cases had fever as the chief complaint. There were 79 (71.8%) patient had history of vomiting and 13 (11.8%) also had rashes.Out of 110 cases enrolled in the study, a total of 92 (83.6%) were flagged as positive by Sysmex.

Table 2: Diagnostic Efficacy of Sysmex Flagging against RDT

Sysmex Flagging	RDT		Total
	Positive	Negative	
Positive	85	7	92
Negative	9	9	18
	94	16	110

{Sensitivity=90.4%; Specificity=56.3%; PPV=92.4%; NPV=50.0%; Accuracy=84.5%
(kappa)=0.444; p<0.001 – Moderate agreement}

RDT detected 94 positive and 16 negative cases. As compared to RDT, flagging was able to detect 85 true positive, 7 false +ve, 9 false -ve and 9 true -ve cases. Correspondingly, the sensitivity, specificity, PPV, NPV and accuracy values of Flagging against RDT were 90.4%; 56.3%; 92.4%; 50.0% and 84.5% respectively.

On evaluating the data statistically, the level of agreement between RDT and flagging was found to be moderate and significant (κ=0.44; p<0.001).

A total of 94 (85.5%) cases were RDT positive and 16 (14.5%) were RDT negative.

Table 3: Peripheral Blood Smear Findings

SN	Findings	No. of cases	Percentage
1.	Positive	99	90.0
	Positive for P. falciparum	9	
	Positive for P. vivax	85	
	Mixed	6	
2.	Negative	11	10.0

{Sensitivity=90.9%; Specificity=81.8%; PPV=97.8%; NPV=50.0%; Accuracy=90%
(kappa)=0.567; p<0.001 – Moderate agreement}
Correspondingly,the sensitivity, specificity, PPV, NPV and accuracy values of Flagging against PBS were 90.9%; 81.8%; 97.8%; 50.0% and 90% respectively.

On evaluating the data statistically, the level of agreement between RDT and flagging was found to be moderate and significant (κ=0.567; p<0.001).

Blood smear positivity rate was 90%. Among 99 positive cases, 85 were positive for P. vivax, 9 were positive for P. falciparum and 6 were positive for mixed infection.RDT detected 94 positive and 16 negative cases. As compared to RDT, flagging was able to detect 85 true positive, 7 false positive, 9 false negative and 9 true negative cases.

On evaluating the data statistically, the level of agreement between RDT and flagging was found to be moderate and significant

DISCUSSION

In the recent years, haematological profile has emerged as a useful indicator for diagnosis of malaria. It has been seen in different studies that haematological parameters are affected during malaria thus indicating, the possibility of use of haematological parameters as possible screening tools for malaria, however, the combination of different haematological tests to suspect malaria is quite complex and is impractical to be used in clinical practice. But in recent years, the automated analyzers have emerged as useful resources performing these complex logical combinations of haematological parameters in order to come up with a flagging for malaria suspect. These analyzers perform analysis of WBC, DLC and parasite with an optical detector block based on flow cytometry method using a semi-conductor laser. A number of previous studies have enumerated the usefulness of flagging for malaria done by automated analyzers.

Age of patients was found out to be between 1 to 70 years. Majority of patient were aged <30 years (n=64; 58.2%) and Mean age of patients was 29.66±17.30 Years. Sharma et al.56 in their study reported the age range of patients between 5 and 60 years and maximum cases in 14 and 25 years range whereas Singh et al.60 enrolled patients aged from 1 to 85 years of age with mean age being 33.6 years.[8,9]

In present study, a total of 92 (83.6%) of the cases were Sysmex positive. Adlekha et al.14 found them to be positive in 24 out of 39 cases (61.5%) whereas Wever et al.48 in their study reported the positivity rate to be as high as 96%. Mubeen et al.49in their study concluded the positivity rate to be 82.2% which is close to that observed in present study. One of the reasons for high positivity rate in present study could be the fact that the present study included only those cases who were positive either by flagging, rapid detection test or blood smear test, thus increasing the domain of positive screen substantially.[10,11]

In present study, peripheral blood smear positivity was observed in 99 (90%) cases. Of these 85 (85.9%) were P. vivax, 9 (9.1%) were P. falciparum and 6 (6.1%) were mixed infections. In various studies from India, a dominance of P. vivax has already been reported. Mubeen et al.49 in their study

had 40/45 (88.9%) P. vivax and 5/45 (11.1%) P. falciparum cases whereas Adlekh et al.14 in their study reported 39 cases had all the cases of P. vivax. Thus the parasitic profile in present study was in concordance with different reports from India.

In present study, we evaluated the sensitivity and specificity of Sysmex flagging against both RDT as well as PBS. We found the sensitivity of flagging to be 90.4% against RDT and 90.9% against PBS. However, the specificity was only 50% against both the modalities. On evaluating the literature, we found that most of the studies have reported a high sensitivity as well as specificity of flagging. Adlekha et al.14 reported it to be 61.5% sensitive and 100 specific and Mohapatra et al. (2011)53 reported it to be 74.2% sensitive and 88% specific and Jain et al. (2012)54 reported it to be 82% sensitive and 100% specific.[12,13]

Thus, although the sensitivity in present study was close to that observed in different studies, however, the specificity for both RDT as well as PBS were drastically low. The reason for this could be the fact that the present study used a screened population for evaluation. In present study, the cases were Sysmex, RDT or PBS positive or had a positive history of confirmed malaria infection in family within last one month, thus the false positive cases lowering the specificity of the evaluation were in effect malaria positive cases either by RDT or PBS. In fact, except for two cases, all the other were positive either by RDT or by PBS and hence the lower specificity of Sysmex when evaluated individually could be justified.

The present educative workout provided a useful insight into the hematological abnormalities in malaria cases. It also showed importance of autoanalyzed flagging in detection of malaria parasite. One of the limitations of the study was that it was carried out in a highly screened population with high probability of positive findings, in such a scenario specificity and negative predictive values have shown to be highly affected while at the same time the sensitivity and positive predictive values are astronomically high.

CONCLUSION

On the basis of observations made in the present study, Age of patients ranged from 1 to 70 years. Majority of patients were aged <30 years (n=64; 58.2%). Mean age of patients was 29.66±17.30 Years.Majority of patients were males (63.6%) and were from suburban/rural areas (81.8%)All the patients presented with fever, vomiting was the next most common complaint (71.8%).

Sysmex flagging was positive in 92 (83.6%) cases. A total of 94 (85.5%) were positive by RDT.PBS positivity was seen in 99 (90%) cases. Among 99 positive cases, 85 were positive for P. vivax, 9 were positive for P. falciparum and 6 were positive for

mixed infection. As compared to RDT, Sysmex flagging had 90.4% sensitivity; 56.3% specificity; 92.4% positive predictive value; 50.0% negative predictive value and 84.5% accuracy for detection of malarial parasite.

As compared to PBS, Sysmex flagging had 90.9% sensitivity; 81.8% specificity; 97.8% positive predictive value; 50.0% negative predictive value and 90% accuracy for detection of malarial parasite.Sysmex flagging had moderate and significant agreement with both RDT and PBS assessments. Flagging had a detection rate of 66.7%, 94.1% and 80% respectively for P. falciparum, P. vivax and mixed infections. On evaluating the data statistically, this difference in detection rate was significant for different species.

The findings of present study showed that Sysmex flagging is a cost-effective highly efficacious tool for screening of malaria parasite and it showed a high level of agreement with conventionally used laboratory tests as well as peripheral blood smear.

The findings of the study showed that despite its usefulness, the specificity of Sysmex flagging was of concern, thus showing that preliminary assessment with flagging should be considered as a screening modality for diagnosis of malaria in all the suspicious cases. In view of no additional cost associated with its use, it can be safely recommended as an additional quick assessment tool comparable to rapid detection tests.

REFERENCES

1. Murray CJL, Lopez AD. Evidence-based health policy— lessons from the Global Burden of Disease Study. Science 1996. 274:740–743.
2. World Malaria Report, 2016. Geneva, Switzerland, 2016. Available at: http://apps.who.int/iris/bitstream/10665/252038/1/9789241511711-eng.pdf
3. Directorate of National Vector Borne Disease Control Programme (NVBDCP). National Framework for Malaria Elimination 2016-2030. Directorate General of Health Services, Ministry of Health and Family Welfare, Government of India, WHO, 2016. Available at: http://www.searo.who.int/india/publications/national_framework_malaria_elimination_india_2016_2030.pdf?ua=1
4. Falade CO, Ajayi IO, Nsungwa-Sabiiti J, et al. Malaria Rapid Diagnostic Tests and Malaria Microscopy for Guiding Malaria Treatment of Uncomplicated Fevers in Nigeria and Prereferral Cases in 3 African Countries. Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America. 2016;63(Suppl 5):S290-S297.
5. World Health Organization. Malaria rapid diagnostic test performance: results of WHO product testing of malaria RDTs: round 6 (2014–2015). Geneva, Switzerland: WHO, 2015.
6. World Health Organisation WHO/HTM/MAL/ 2006.1111: The role of laboratory diagnosis to support malaria disease management: Focus on the use of rapid diagnostic tests in areas of high transmission. Report of WHO technical consultation.WHO Geneva; 2006.
7. Adlekha S, Jaiswal RM, Chadha T, Singla A. The correlation of spurious eosinophilia in automated hematological analyzer

Sysmex XS-800i with Plasmodium infection diagnosis.Indian J Med Sci. 2011 Nov;65(11):469-75.

8. Sharma S, Sethi N, Pujani M, Kushwaha S, Sehgal S. Abnormal WBC scattergram: a clue to the diagnosis of malaria. Hematology. 2013 Mar;18(2):101-5. Link

9. Singh A, Narang V, Sood N, Garg B, Gupta VK. Malaria Diagnosis Using Automated Analysers: A Boon for Hematopathologists in Endemic Areas. Journal of Clinical and Diagnostic Research : JCDR. 2015;9(10):EC05-EC08. Link

10. Wever PC, Henskens YMC, Kager PA, Dankert J, van Gool T. Detection of Imported Malaria with the Cell-Dyn 4000 Hematology Analyzer. Journal of Clinical Microbiology. 2002;40(12):4729-4731. Link

11. Mubeen KH, Devadoss CW, Rangan RA, Gitanjali M, Prasanna S, Sunitha V. Automated Hematology Analyzers in Diagnosis of Plasmodium vivax Malaria: an Adjunct to Conventional Microscopy. Mediterranean Journal of Hematology and Infectious Diseases. 2014;6(1):e2014034. Link

12. Mohapatra S, Samantaray JC, Arulselvi S, Panda J, Munot K, Saxena R. Automated detection of malaria with haematology analyzer Sysmex XE-2100. Indian J Med Sci 2011;65:26-31. Link

How to cite this article: Ayyub SA, Kumar A, Dutta S, Joshi H. Flagging Of Malaria Parasite In Hematology Analyzer With Its Correlation On Peripheral Smear And Rapid Diagnostic KIT. Ann. Int. Med. Den. Res. 2018; 4(2):PT10-PT14.

Source of Support: Nil, **Conflict of Interest:** None declared