

## Utility of automated hematology analyzer in diagnosis of malarial parasite

Alpesh M. Maru<sup>1</sup>, Atul Shrivastava<sup>2\*</sup>, Tejas Chokshi<sup>3</sup>, A.S. Agnihotri<sup>4</sup>

<sup>1</sup>Assistant Professors, <sup>2</sup>Associate Professor, <sup>3</sup>Professor, <sup>4</sup>Professor and Head, Dept. of Pathology, GMERS Medical College, Junagadh, Gujarat, <sup>2</sup>GMERS Medical College, Himmatanagar, Gujarat, <sup>3,4</sup>C.U. Shah Medical College, Surendranagar, Gujarat, India

### Article Info

**Received:** 25<sup>th</sup> October, 2018

**Accepted:** 29<sup>th</sup> January, 2019

**Published Online:** 9<sup>th</sup> August, 2019

**Keywords:** Malaria, Scattergram, Pseudo eosinophilia.

### Abstract

**Introduction:** Malaria is the most common infectious disease that affecting many people worldwide. Microscopic method is remain gold standard method for diagnosis but Hemazoin pigments produced by malaria are also scatter the laser light and producing abnormal scattergrams during CBC analysis & thus we have conducted this study to prove usefulness of 5-part automated hematology analyzer (LH-750) in presumptive diagnosis of malaria

**Materials and Methods:** This prospective study was done at pathology department of C.U. Shah Medical college & hospital, Surendranagar from November 2013 to April 2014. 2 ml of EDTA blood samples were collected and blood analysis was done on hematology analyzer (5-part LH – 750). Thick and thin blood films were prepared and stained with giemsa stain in all cases.

**Results:** Among 100 positive cases out of 2710 samples, 63 cases were positive for plasmodium vivax and 37 cases were positive for plasmodium falciparum. Among 100 malaria positive cases, abnormal scattergram pattern was observed in 65 cases. In P.vivax group (n=63 cases), 50 cases showed abnormal scattergram out of 63 cases, having sensitivity of 79.36%. In P. falciparum group (n=37 cases), 15 cases showed abnormal scattergram out of 37 cases with having sensitivity of 40.54%. Various abnormalities found on WBC-DIFF scattergrams in patients of malaria were merging & graying of neutrophils & eosinophils clusters, prominent blue coded events below neutrophils clusters.(in RBC ghost region)

**Conclusion:** Automated hematology analyzer is very important diagnostic tool for malaria with scattergram abnormalities are helpful in presumptive diagnosis of malaria. All such cases should be confirmed by gold standard method.

### Introduction

Malaria is the most common infectious disease that affecting many people worldwide. It is the leading public health problem all over the world especially countries like india.<sup>1</sup> Malaria is the leading cause of death due to parasitic infection in tropical & subtropical countries.<sup>2</sup> Clinically patients of malaria are present with typical paroxysm of malaria like fever with chills & rigors followed by sweat and perspiration. Diagnosis of malaria can be done by clinical history and laboratory tests.

Microscopic method like PS for MP (Peripheral Smear for Malaria Parasite) for detection of malaria is remain gold standard method for diagnosis of malaria till date. It is very cost effective and accurate method but having demerit of having high degree of subjective variations. Correct reporting of blood films required good expertise and it is also less reliable method when there is low parasitic index.

So some alternative rapid diagnostic tests by immunochromatography as well as QBC (Quantitative Buffy Coat) tests are being done now a days. Although they

are expensive and however they are not routinely available everywhere.<sup>3,4</sup>

CBC (complete blood count) test is most common laboratory investigation prescribed in almost all cases of fever with chills & rigors. CBC is performed by using automated hematology analyzer. The hematology analyzer works on basic principle of flow cytometry by VCS [Volume, Conductivity, Scattering] technology & producing scattergrams.<sup>5</sup> Automated hematology analyzer can differentiate WBCs by using side fluorescence and side scattered light.

Malarial parasite usually produce Hemazoin pigment which has property to scatter the laser light and producing various abnormal scattergrams during routine CBC analysis.<sup>6</sup> We aim to conduct this study to prove usefulness of 5-part automated hematology analyzer (LH-750) in presumptive diagnosis of malaria along with peripheral smear examinations.

\*Corresponding Author: Atul Shrivastava, Associate Professor, Dept. of Pathology, <sup>2</sup>GMERS Medical College, Himmatanagar, Gujarat, India

Email: [drmaru28@gmail.com](mailto:drmaru28@gmail.com)

<http://doi.org/10.18231/j.ijpo.2019.082>

## Materials and Methods

This prospective study was carried out at pathology department of C.U. Shah Medical college & hospital, Surendranagar. Blood samples from both out patients & indoor patients were analyzed from November 2013 to April 2014, six months study. Patients with fever & chills of all age groups and both sexes were included in the study (n=2710).

2 ml of EDTA blood samples were collected and blood analysis was performed on hematology analyzer (5-part LH – 750). Thick and thin blood films were prepared and stained with giemsa stain in all cases.

Hematology analyzer uses flow cytometry as a principle and by using semi-conductor diode laser light at 90° for side scatter (ssc) view & at 0° for forward scatter (fsc). Forward scatter light analyze the size of WBCs and Side scatter light analyze granularity of WBCs and thus producing WBC-DIFF scattergrams.

Various patterns seen in WBC-DIFF scattergram were analyzed and at the same time microscopic examination of smears were done to compare and match the data with abnormal scattergrams. In this study, positive case of malaria was confirmed either by positive Giemsa stained P/S or by rapid diagnostic malaria antigen card test. Samples were considered as negative only after examining and checking that not a single ring form, trophozoites, schizontes or gametocytic form of parasite seen in at least 200 fields of thin smears under oil immersion.

Various other hematological parameters like Haemoglobin(Hb), Platelet count, Differential count(DC) and Pseudo eosinophilia were analyzed for all such malaria positive cases.

Sensitivity, specificity, PPV (positive predictive value) & NPV (negative predictive value) were calculated for WBC-DIFF scattergrams of all above samples and compared with other hematological parameters.

Statistical analysis was performed with the help of SPSS (statistical package for social science) software & P value of < 0.05 were considered as statistically significant.

## Observations and Results

In present study, we have received 2710 blood samples of patients suffering from febrile illness. Study duration was from November 2013 to April 2014. Out of 2710 cases, 100 cases (n=100) were found to be positive for plasmodium species by either Giemsa stained P/S or rapid diagnostic tests.

Out of 100 positive malaria cases, 65 males were affected and 35 females were affected with age range vary from 10 to 65 years. Among 100 positive cases out of 2710 samples, 63 cases were positive for plasmodium vivax and plasmodium falciparum. Positivity observed in 37 cases. (Table 1) Mean age of p. vivax patients was 30.04 years (Male – 28.93 years, Female – 31.95 years) while Mean age of p.falciparum patients was 32.85 years (Male – 32.22 years, Female – 34.16 years).

**Table 1:** Showing Mean age & sex wise distribution of malaria species.

	P. Vivax (63 cases)		P. Falciparum (37 cases)	
	Male	Female	Male	Female
Total positive cases (100)	40	23	25	12
Mean Age	28.93	31.95	32.22	34.16

## WBC-DIFF Scattergram Findings

On 5-part hematology analyzer, WBC-DIFF scattergrams were plotted and analyzes in each 2710 cases. Out of 100 malaria positive cases, 65 cases were found to show abnormal scattergram. In P.vivax group (n=63 cases), 50 out of 63 cases showed abnormal scattergram (Table – 2) with sensitivity of 79.36%. In P. falciparum group (n=37 cases), 15 cases showed abnormal scattergram out of 37 cases with having sensitivity of 40.54%. Specificity for both P. vivax and P. falciparum on WBC-DIFF scattergram was 93.54%. Positive predictive value (PPV) for P.vivax was 21.73% and for P.falciparum 7.69% respectively while Negative predictive value (NPV) was 99.50% & 99.16% for P. vivax and P. falciparum respectively. (Table 3)

**Table 2:** Showing distribution of malaria cases as per WBC-DIFF scattergrams

Abnormal Findings on WBC-DIFF scattergram	Malaria Positive cases(n=100)				Malaria Negative cases(n=2610)	
	P.vivax(63)		P.falciparum(37)		True Negative(TN)	False Positive(FP)
	Positive	Negative	Positive	Negative		
	50(TP)	13(FN)	15(TP)	22(FN)	2610	180

TP: True Positive, FN: False Negative, TN: True Negative, FP: False Positive

**Table 3:** Showing sensitivity, specificity, PPV, NPV of WBC-DIFF scattergrams for malaria detection.

	Sensitivity	Specificity	Positive predictive value(PPV)	Negative predictive value(NPV)
P. Vivax	79.36%	93.54%	21.73%	99.50%
P. falci	40.54%	93.54%	7.69%	99.16%

Normal data plot and scattergram images of backman coulter, LH-750 hematology analyzer has been shown in Fig. 1A, 1B.

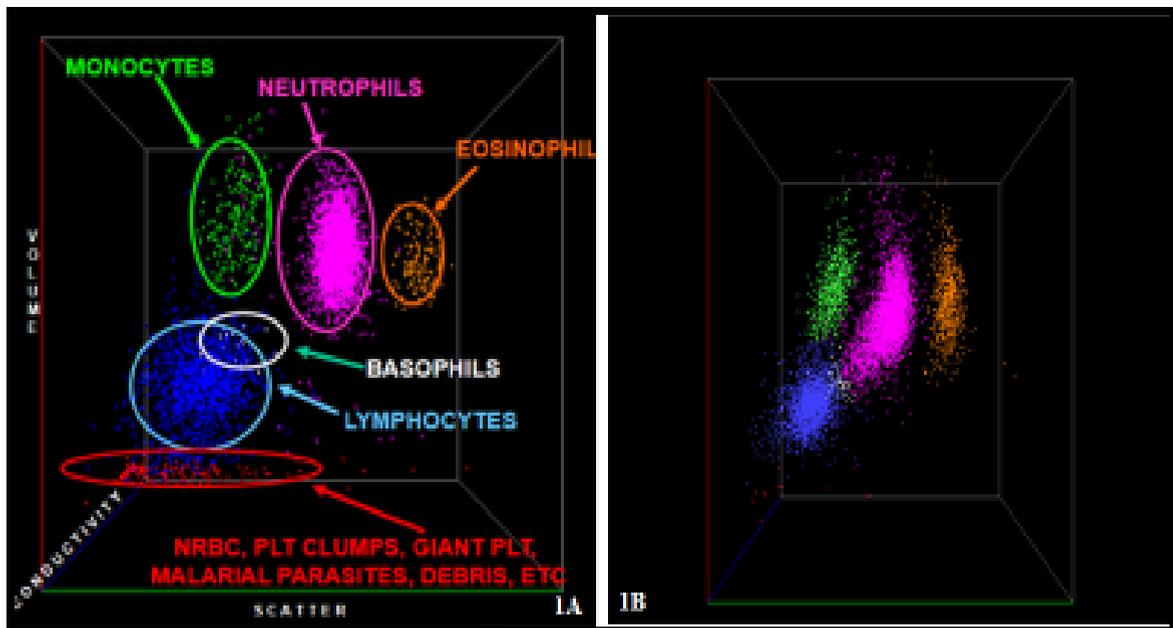


Fig. 1A,1B: Showing Normal distribution of scatter gram plotted in LH-750 Analyzer

Various abnormal patterns found on WBC-DIFF scattergrams were merging of neutrophils & eosinophils clusters, multiple neutrophils or eosinophils clusters, graying of neutrophils & eosinophils clusters, decreased space between eosinophils & neutrophils clusters, large eosinophils clusters and prominent blue coded events below neutrophils clusters.(in RBC ghost region)

Fig. 2A, 2B shows abnormal P.vivax scattergrams presenting RBC ghost region below neutrophils and merging of neutrophils and eosinophils clusters on WBC-DIFF scattergrams. Fig. 3A, 3B shows abnormal P.falciparum scattergrams showing prominent ghost region and graying of neutrophils & eosinophils clusters.

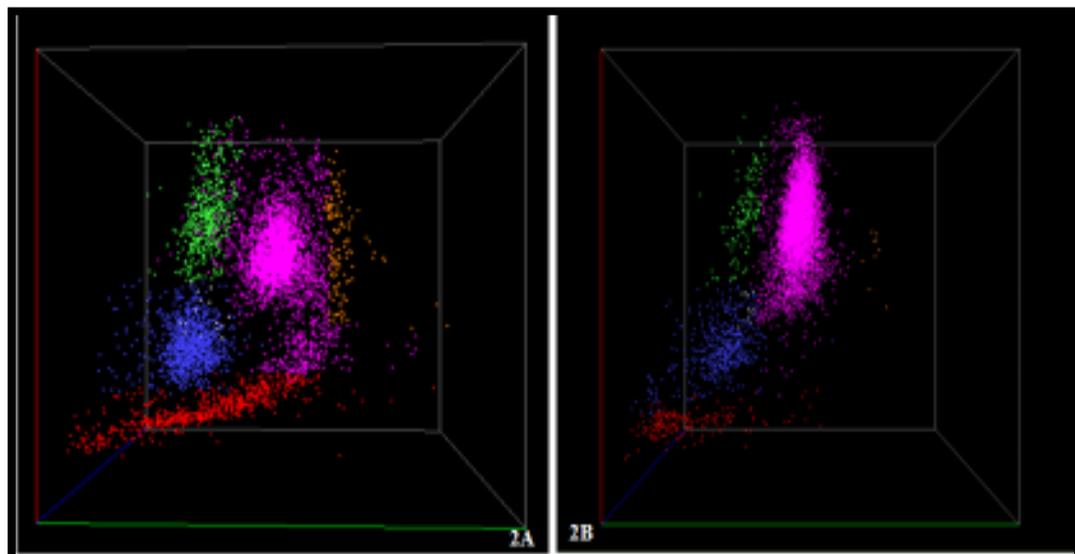
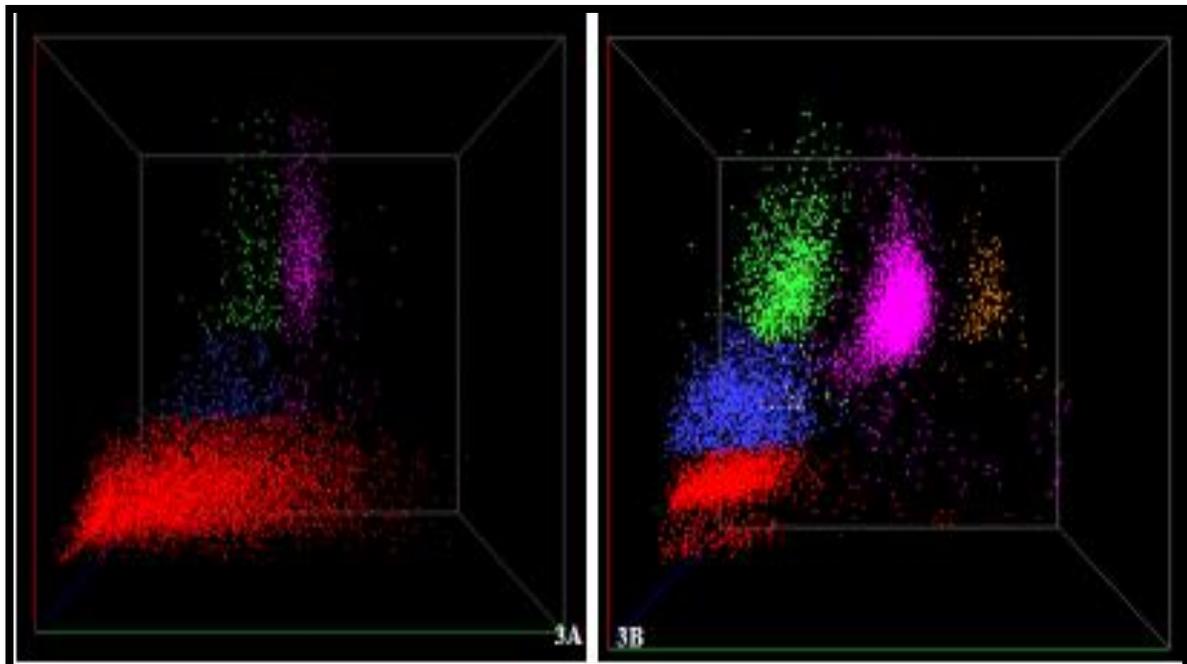


Fig. 2A, 2B: Shows abnormal scatter gram in case of P. Vivax having RBC Ghost region below Neutrophils and merging of Neutrophils and Eosinophils (WBC/DIFF Scatter gram)



**Fig. 3A, 3B:** Shows abnormal scatter gram in case of *P. Falciparum* having Prominent Ghost region & greying of Neutrophils and Eosinophils (WBC/DIFF Scatter gram)

**Table 4:** Showing sensitivity & specificity of WBC-DIFF scatter grams for detection of *P. Vivax*.

	Kolakkadan et al <sup>[10]</sup>	Pai vidya et al <sup>[11]</sup>	Zuluaga et al <sup>[12]</sup>	Huh et al <sup>[13]</sup>	Yoo et al <sup>[14]</sup>	Jain et al <sup>[15]</sup>	Present study
Sensitivity	87.50%	76%	97%	52%	46.2%	82%	79.36%
specificity	92.93%	67%	94%	100%	99.7%	100%	93.54%

### Various Hematological Parameters and Malaria

The hematological parameters such as Platelets, Hemoglobin & Total leukocyte count (TLC) were decreased in malaria positive patients as compared to malaria negative patients. Majority patients of malaria present with low hemoglobin, thus anemia was common findings in such all patients. Out of 100 cases, 93 patients (93%) were found to be anemic.

Thrombocytopenia was observed in 89 out of 100 malaria positive patients (89%) with platelets were ranging from 10,000 to 1,50,000/cu mm.

Leukopenia was relatively common findings in patients with malaria and among differential count, lymphopenia was observed in 31 cases out of 100. (31%) Lymphopenia is due to transiently decrease number of circulating lymphocytes in malaria by either destruction or redistribution of lymphocytes.

Pseudo eosinophilia is defined as a difference between automated hematology analyzer findings and manual eosinophil count  $\geq 5\%$ . In our study 13 cases (13%) were reflecting pseudo eosinophilia.

### Discussion

Malaria is very common parasitic disease and major health problem worldwide. Now a days many effective ways for management of malaria are exist. On the other hand, current

scenario suggest that number of malaria cases are still increasing.

Malaria is caused by plasmodium parasites. It is a vector born disease (female anopheles) & having various subtypes like *P.vivax*, *P. falciparum*, *P.ovale* & *P.malariae*. Malaria is highly prevalent in tropical and subtropical countries. So it is necessary to establish rapid, cost effective method for screening and diagnosis of malaria from blood samples.

Peripheral smear for malaria by giemsa stain is remain gold standard for diagnosis of malaria. Thick smear is useful in conditions with low parasitemia & thin smear is indicated for species confirmation.

Various newer modalities for diagnosis of malaria are QBC (Quantitative Buffy Coat) assay, Antigen dipstick test, Rapid diagnostic card test & PCR (polymerase chain reaction) have been used in recent past.<sup>7,8</sup> however QBC method has certain disadvantages like high cost of equipment & non specific nature of acridine orange dye as Howell-jolly bodies also take up the stain. PCR is also sensitive but again it is expensive.

CBC test is done routinely in almost all blood samples of febrile patients. Utility of automated hematology analyzer for diagnosis of malaria have been reported in many literature. The Cell-Dyn 3500 was first analyzer that used to detect malaria.<sup>9</sup>

LH-750 hematology analyzer uses VCS technology and flow cytometry in conjunction with fluorescence properties of WBCs & generate various scattergrams. The Hemazoin pigments liberated from break down of RBC. These pigments are than engulfed by neutrophils, monocytes and macrophages but these pigments are birefringent in nature. Thus it is capable of scattering laser light and form abnormal scattergrams. Analyzer identifies parasites and based on their size and pigment contents, they appear in WBC-DIFF plots in areas of neutrophils, eosinophils clusters.

Similar study was conducted by kolakkadam et al,<sup>10</sup> in their study out of 2610 febrile patients, 45 cases showed smear positivity & among them 40 cases were infected by P. vivax & 05 cases were of P. falciparum. On WBC-DIFF scattergrams, there were abnormal plots observed in 37 patients out of 45 positive cases with sensitivity for P.vivax & P. falciparum was 87.50% & 40% respectively, while specificity was 92.93% in both P.vivax and P.falciparum cases. Positive predictive value (PPV) for P. vivax was 16.13% and for P.falciparum 1.09%. Negative predictive value (NPV) was 99.79% & 99.87% for P.vivax and P. falciparum respectively. Similar findings were observed in our study.

Other study like, Pai Vidya et al<sup>11</sup> showing 76% sensitivity & 67% specificity with 179 cases shows abnormal scattergrams out of 256 malaria positive cases. Findings quite correlate with our study.

Study of Zuluaga et al<sup>12</sup> shows 97% sensitivity and 94% specificity on WBC-DIFF scattergrams. Study of Huh et al<sup>13</sup> shows abnormal scattergrams for P. Vivax with sensitivity of 52% & specificity of 100%. Study of Yoo et al<sup>14</sup> & study of Jain et al<sup>15</sup> again show similar results that correlated well with our study.(Table 4)

Thrombocytopenia was observed in 89% cases in our study, which was similar to findings of study of Arbo et al<sup>16</sup> & Chandra et al.<sup>17</sup> In both their study they had observed 97% cases of thrombocytopenia with malaria positivity.

In present study, pseudo eosinophilia was found in 13% cases. Similar findings were observed in study of Huh et al<sup>13</sup> with 38% cases having pseudo eosinophilia, kolakkadan et al<sup>10</sup> having 15% & Zuluaga et al<sup>12</sup> having 38% cases with pseudo eosinophilia. Such findings are correlated well with our study.

Apart from WBC-DIFF abnormal scattergrams, various other abnormalities of hematological parameters observed in our study are leukopenia, lymphopenia, anemia & abnormal platelets distribution in patients of malaria. Similar hematological findings were observed in study of Pai vidya et al,<sup>11</sup> Jain et al,<sup>15</sup> & Abdall et al.<sup>18</sup>

## Conclusion

Automated hematology analyzer is very important diagnostic tool for malaria detection even in the absence of any requisition from clinical side, minimizing chances of false negative error.

It is recommended to all pathologists to review scattergrams produced by cell counter in all suspected cases.

Thus WBC-DIFF scattergram abnormalities are useful in presumptive diagnosis of malaria along with other abnormal hematological parameters such as thrombocytopenia, pseudo eosinophilia, leukopenia.

All such cases should be confirmed by gold standard method (PS for MP) and by various method like rapid diagnostic malaria tests.

**Conflict of Interest:** None.

**Source of Funding:** None.

## References

1. Chipeta J, Mharakurwa S, Thuma P, Kumar N. A synopsis of current malaria diagnosis trends. *Med J Zambia* 2009;36(2):95-101.
2. World malaria report 2013. Geneva, World Health Organization 2013. ([http://www.who.int/malaria/publications/world\\_malaria\\_report\\_2013/en/](http://www.who.int/malaria/publications/world_malaria_report_2013/en/)).
3. Acharya AR, Magisetty JL, Chandra A, Chaithra BS, Khanum T, Vijayan VA. Trend of malaria incidence in the state of Karnataka, India for 2001 to 2011. *Archives of applied science research*.2013; 5(3):104-11.
4. Sharma S, Sethi N, Pujani M, Kushwaha S, Sehgal S. Abnormal WBC scattergram: A clue to the diagnosis of malaria. *Hematol* 2012;18(2):101-5.
5. deGroot BG, Terstappen LW, Puppels GJ, Greve J. Light-scattering polarization measurements as a new parameter in flow cytometry. *Cytometry* 1987;8:539-44.
6. Huh HJ, Jung J, Yoon H, Chae SL. Malaria detection with the Sysmex XE-2100 hematology analyzer using pseudo eosinophilia and abnormal WBC scattergram. *Ann Haematol* 2008;87:755-9.
7. Bailey JW, Williams J, Bain BJ, William JP, Chiodin PL. Guideline: the laboratory diagnosis of malaria. *Br J Haematol* 2013;163:573-80.
8. Yan J, Li N, Wei X, Li P, Zhao Z, Wang L et al. Performance of two rapid diagnostic tests for malaria diagnosis at the China Myanmar border area. *Malaria J* 2013;12:73.
9. Hanscheid T, Pinto BG, Pereira I, Cristino JM, Valadas E. Avoiding misdiagnosis of malaria: A novel automated method allows specific diagnosis, even in the absence of clinical suspicion. *Emerg Infect Dis* 1999;5:836-38.
10. Kolakkadan Hasaf Mubeen, Clement Wilfred Devadoss, Rau Aarathi Rangan, Monnappa Gitanjali et al. Automated Hematology Analyzers in Diagnosis of Plasmodium vivax Malaria: An adjunct to Conventional Microscopy. *Mediterr J Hematol Infect Dis* 2014;6(1):e2014034.
11. Pai Vidya, Shariff M.H. and Aijaz Muzamil Dar. Malaria diagnosis by abnormal scattergrams in automated hematology analyzer. *Int J Pharmacy Biological Sci* 2016;6(2):55-9.
12. Campuzano-Zuluaga G, Alvarez-Sánchez G, Escobar-Gallo GE, Valencia-Zuluaga LM, Ríos-Orrego AM, Pabón-Vidal A, et al. Design of malaria diagnostic criteria for the Sysmex XE-2100 hematology analyzer. *Am J Trop Med Hyg* 2010; 82:402-11.
13. Huh HJ, Oh GY, Huh JW, Chae SL. Malaria detection with the Sysmex XE-2100 hematology analyzer using pseudo eosinophilia and abnormal WBC scattergram. *Ann Hematol* 2008;87:755-9.
14. Yoo JH, Song J, Lee KA. Short Report: Automated detection of malaria-associated pseudo eosinophilia and abnormal wbc scattergram by the sysmex xe-2100 hematology analyzer: a clinical study with 1,801 patients and real-time quantitative PCR

- analysis in vivax malaria endemic area. *Am J Trop Med Hyg* 2010;82:412-4.
15. Jain M, Gupta S, Jain J, Grover RK. Usefulness of automated cell counters in detection of malaria in a cancer set up-Our experience. *IJPM* 2012;55(4):467-73.
  16. Abro AH, Saleh AA, Abdou AS, Ustadi AM, Shuri M, Seliem RM. Thrombocytopenia in adults with acute malaria. *RMJ* 2009;34(2):170-2.
  17. Chandra S, Chandra H. Role of Haematological Parameters as an Indicator of Acute Malarial Infection in Uttarakhand State of India. *Mediterr J Hematol Infect Dis* 2013;5(1): e2013009.
  18. Abdulla SH. Leukocytes in Malaria. In: Abdulla SH, Pasvol G. Malaria-A Hematological perspective. Tropical Medicine Science and practice. Vol 4. London: Imperial College press;2004:129-68.

**How to cite this article:** Maru AM, Shrivastava A, Chokshi T, Agnihotri AS. Utility of automated hematology analyzer in diagnosis of malarial parasite. *Indian J Pathol Oncol* 2019;6(3):428-33.