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Comparative Malaria Diagnosis Using LH750 Beckman Coulter Automated Analyser in India: A Cross Sectional Case Control Study

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Objective: To study the VCS pattern in patients with malaria and compare it with patients having fever who were negative for malarial parasite (*P. falciparum, vivax, malariae and ovale*) and normal individuals and to determine whether a further adaptation of the discriminant factor is needed using additional parameters from the VCS data analysis using Beckmann Coulter LH 750.
Design: Cross sectional Case control study.
Setting: Clinical Laboratory and Haematology Department, KMC, India.
Patients: Ninety-three Malaria positive samples and fifty malaria negative samples by QBC method comprised the Case and hundred healthy adult samples formed the Control group.
Interventions: All blood samples were analysed using LH750 Beckman Coulter for VCS data of the neutrophils, lymphocytes, monocytes and eosinophils and compared between Malaria positive, Malaria negative and Control Group.
Main Outcome Measure: Discriminant Factor.
Results: The difference in SD volume of the lymphocyte and monocyte among malaria positive and

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negative were statistically significant. By using logistic regression, the “Malaria factor” is defined. ROC analysis showed using a cut-off value of 2.7 as malaria factor derived from SD volume, specificity of 92.5 and sensitivity of 83.7 with a PPV of 88.6% and NPV of 89.1% were obtained whereas, specificity and sensitivity obtained using SD conductivity (91 and 75.5 respectively) and SD scatter (68.7 and 57.1 respectively) were significantly less.

Conclusions: A “suspect malaria” flag could be generated on the analyser, allowing the detection of cases of unsuspected malaria and therefore, early diagnosis of the disease with the potential of reducing the possibility of serious complications however this is not a confirmative test.

Keywords: Malaria; malaria diagnosis; automated analyser; coulter; malaria factor.

1. INTRODUCTION

Malaria is a major health problem with over 40% of world’s population exposed to varying degree of malaria risk. India is endemic to malaria with a case load of 1.49 million in 2010 [1]. WHO estimated 216 million cases in 2016 with a five million increase in global incidence of Malaria as compared to 2015 [2]. Cornerstones in global malaria control strategy are effective disease management by prompt and accurate diagnosis. Earliest symptoms of malaria are nonspecific and hence the clinical diagnosis is challenging and not reliable as it overlaps with most of the viral diseases and febrile illnesses [3,4].

Despite good sensitivity and specificity, all malaria tests including the new ones have the inherent disadvantage that they have to be requested explicitly and lack of clinical suspicion have been reported as one of the main reasons for misdiagnosis [5]. Parasitological diagnosis of malaria is by Giemsa-stained Thick blood Film (GTF) that help in speciation and diagnosis. Other methods like Quantitative Buffy Coat (QBC) and Rapid Diagnostic Tests (RDT) are not highly sensitive and thus not reliable in detection in low-density malaria infections. Recombinase polymerase amplification (RPA) is a new mode of diagnosis with high specificity. However, it is costly and is not being used extensively. Hence, the need for a sensitive and reliable test using laboratory technology.

Automated analysers uses VCS technology to quantify WBCs, it can also detect the infected hemozoin ingested WBC by the difference in volume conductivity and scatter as this has a different scatter plot and this can be used as a screening method especially in endemic area [6,7]. Malaria detection in previous studies by automation used a discriminant factor derived from differences in standard deviation (SD) of volume of lymphocytes and monocytes [8,9]. Factor was different in each study and varied with each species of plasmodium. We analysed the VCS pattern in patients with malaria and compared with malaria parasite negative fever patients and normal population to deduce a Malaria factor that can be used in Indian population to detect the possibility of infection. This study gains importance by being conducted in South Karnataka, which is Malaria endemic.

1.1 Objectives

1. To study the VCS pattern in patients with malaria and compare it with patients with fever who were malaria parasite negative and with normal individuals.
2. To evaluate the feasibility of automated malaria detection using a defined Discriminant Factor and to determine whether a further adaptation of the discriminant factor is needed using additional parameters from the VCS data analysis using Beckmann Coulter LH 750.

2. METHODS

The study area (Udupi District) is endemic for malaria infection with 1188 cases reported in 2010, of which 194 were *P. falciparum* infection. Our study was a Cross-sectional Case control study with the blood samples collected from patients attending Hospital with fever or clinically suspected malaria during the study period 2 months from July 2011 to August 2011. Institute Ethical Committee had approved the study IEC231/2011. Samples collected from adult individuals were grouped into three groups.

- Group 1: Patient with fever and diagnosed as Malarial Parasite positive by fluorescent method (Quantitative buffy coat) and/or Immunochromatography. Ninety-three patient sample during the study period formed the study group.

- Group 2: Fifty samples of patient with fever and diagnosed as Malarial Parasite negative by fluorescent method (Quantitative buffy coat) and/or Immunochromatography.
- Group 3: Hundred samples from apparently healthy adults in whom all CBC parameters were within reference range and from voluntary blood donors formed the Control group.
- Patients with previously diagnosed systemic diseases presenting with fever were not included in the study. We ensured precision of VCS data since we run two level controls for CBC & differential count twice daily on Beckman Coulter LH 750.

The quantitative buffy coat method (QBC) combines an acridine orange coated capillary tube, an internal float, and separates layers of Platelets, WBCs and RBCs using centrifugation. Parasites concentrate below the buffy coat and appear in the upper layer of RBCs. The parasites can be seen when the tube is examined under a UV light source [10].

2.1 Protocol

We collected all peripheral blood samples in K_3EDTA tubes and analysed within 2 hours using LH750 Beckman Coulter for VCS data. We recorded the VCS data of the neutrophils, lymphocytes, monocytes and eosinophils and compared between Malaria positive, Malaria negative and Control Group. In Malaria positive samples, there was volume heterogeneity in lymphocytes and monocytes.

2.2 Variables

Outcome variable is Malaria detection. Dependant variables are Volume, conductivity and scatter data. Confounding factors are age and gender.

2.3 Study Size

All malaria positive patient samples during the study period July 2011 to August 2011 (2 months), which was 93 cases comprised the study group. Fifty Malaria negative samples confirmed by QBC/Immunochromatography and hundred samples from apparently healthy individuals formed the control group.

2.4 Statistical Analysis

Data was entered and analysed using SPSS (version 15, SPSS South Asia Bangalore).The continuous variables were analysed using mean and standard deviation. Comparison of the continuous variables between the patient and normal subjects were done using one-way analysis of variance. By logistic regression Malaria factor was calculated as:

Malaria factor = exp (-13.880+ (0.246*lymphocyte_SD)+(0.475*monocyte_SD))

Receiver operating characteristic (ROC) analysis was performed on the malaria factor to determine whether a satisfactory cut-off value could be established for the detection of malaria in a blood sample. The results of the SD volume, conductivity & scatter of the neutrophils, lymphocytes, monocytes & eosinophils were analysed by using the Kruskal Wallis test to determine any statistical differences between three groups.

3. RESULTS

During the 2 months study period, we found 93 cases to be malaria positive among the suspected cases. All malaria positive cases out of the total 243 samples, had volume heterogeneity in lymphocytes and monocytes as compared to malaria negative cases and normal samples. Table 1 shows the range of Standard deviation (SD) of lymphocyte and monocyte observed in the study. The difference in SD for the variables among all 3 groups were statistically significant (p 0.000) for SD volume of neutrophil, lymphocytes, monocytes, SD conductivity of neutrophil, lymphocyte, monocyte, eosinophil, SD scatter of neutrophil and lymphocyte as shown in Table 2.

On comparing the significant difference in mean volume, conductivity and scatter between malaria positive, malaria negative and control groups, SD volume of Monocytes and Lymphocytes among malaria positive cases were significantly different from negative and control group (Table 3). Malaria factor was calculated using logistic regression. We did ROC analysis for the malaria factor thus calculated from the equation using SD of Volume, Conductivity and Scatter. Fig. 1 shows the ROC curve of Malaria factor calculated from the SD volume, Conductivity and Scatter. Table 4 summarises the findings of the

ROC analysis between volume, conductivity and scatter.

True positive, true negative, false positive and false-negative results obtained in Volume, Conductivity and Scatter are given in Table 5. These figures were used to calculate the positive predictive value (PPV) and negative predictive value (NPV) for this study. From the findings, the Malaria Factor obtained using the SD volume difference in lymphocyte and monocyte has the highest specificity and sensitivity of 92.5 and 83.7 respectively with a PPV of 88.6% and NPV of 89.1%.

Table 1. Range of SD volume of lymphocyte and monocyte

Volume	Positive	Negative	Control
Lymphocyte SD	9.32 - 32.94	10.32 – 24.79	11.06 – 15.85
Monocyte SD	18.99 - 42.51	16.86 – 41.57	14.84 – 23.6

SD=Standard Deviation

Table 2. Kruskal Wallis test result for SD volume scatter and conductivity

Variable		p Value
Volume	Neutrophils	**.000**
	Lymphocytes	**.000**
	Monocytes	**.000**
	Eosinophils	**.111**
Conductivity	Neutrophils	**.000**
	Lymphocytes	**.000**
	Monocytes	**.000**
	Eosinophils	**.001**
Scatter	Neutrophils	**.000**
	Lymphocytes	**.000**
	Monocytes	**.103**
	Eosinophils	**.076**

SD= Standard Deviation

The Kruskal Wallis test result for the SD volume scatter and conductivity

Table 3. Mann Whitney U test comparing malaria positive, negative and control group

Variable		Positive & negative	Positive & control	Negative & control
Volume	Neutrophil	.183	.000	.000
	Lymphocyte	.000	.000	.048
	Monocyte	.000	.000	.000
	Eosinophil	.002	.130	.014
Conductivity	Neutrophil	.001	.203	.009
	Lymphocyte	.793	.000	.000
	Monocyte	.700	.000	.000
	Eosinophil	.573	.000	.000
Scatter	Neutrophil	.000	.000	.856
	Lymphocyte	.708	.000	.000
	Monocyte	.189	.068	.002
	Eosinophil	.029	.004	.491

Lymphocyte and Monocyte Standard deviation of volume were significantly different among all three groups

Table 4. ROC analysis comparison among volume, conductivity and scatter

	Volume	Conductivity	Scatter
Cutoff value	2.7	0.4	0.54
Area under curve	0.891	0.883	0.710
Specificity	92.5%	91.0%	68.7%
Sensitivity	83.7%	75.5%	57.1%

ROC=Receiver Operator Curve

Table 5. Comparison of true positive, true negative, false positive, false negative, PPV and NPV of the study

	Volume	Conductivity	Scatter
True-positive	62	61	46
True-negative	41	37	28
False-positive	8	12	21
False-negative	5	6	21
PPV	88.6%	83.6%	68.7%
NPV	89.1%	86%	57.1%

PPV=Positive Predictive Value; NPV= Negative Predictive Value

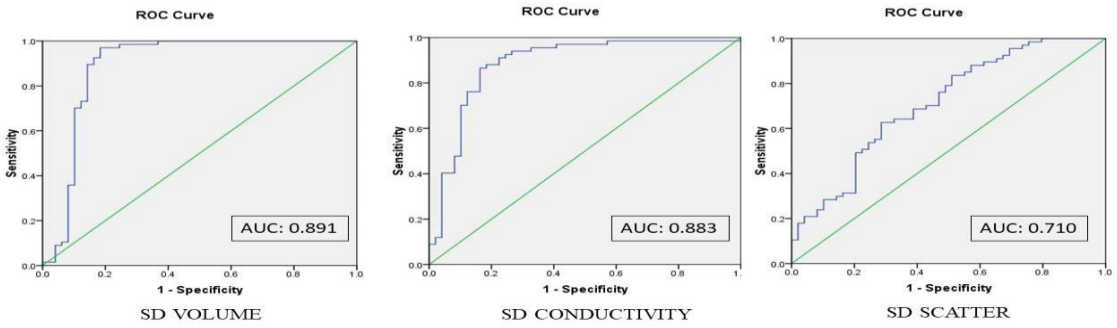

Figure 1: Comparison of ROC curve of Malaria factor calculated from the SD volume, SD Conductivity and SD Scatter.

SD = Standard Deviation; ROC= Receiver Operator Curve; AUC= Area Under Curve

Fig. 1. Comparison of ROC of malaria factor calculated from SD volume, SD conductivity and SD scatter ROC

4. DISCUSSION

Malaria is not only a health issue for the developing country but an economic burden. It is one of the most common parasitic diseases in the world. Morbidity of the disease is attributed to delay in diagnosis, the mainstay of which is clinical suspicion. The “gold standard” diagnostic modality for malaria is still Giemsa-stained Thick blood Film (GTF), microscopic examination of stained thick and thin blood films. The disadvantage being it is difficult, time-consuming and low levels of parasitemia may be missed [11].

Quantitative Buffy Coat (QBC) technique is used as a backup to blood films and to simplify malaria diagnosis [12]. The overall sensitivity rate for QBC in a study by Adeoye et al. [13] was 55.9% when compared with GTF. Sensitivity of QBC and GTF reduces further with samples having low parasite density. QBC test cannot differentiate accurately between different Plasmodium species but with the GTF. In spite of the speed and simplicity of QBC technique, it cannot be considered an acceptable alternative to GTF under routine clinical laboratory situation. The disadvantages of this method are the high cost of the equipment, nonspecific nature of stain and consumables. Many rapid methods are present in the market with variable detection rate, which still needs clinical suspicion to be asked for. High false positivity in high WBC counts or in leukemic patients, persisting histidine rich proteins for days after treatment, inability to detect P.ovale and malariae, prozone effect and inability to quantify adds as the disadvantage for these methods [14]. Polymerase chain reaction has been proven to be sensitive in the diagnosis of all four species of malaria. However, it is expensive and impractical in the routine diagnosis of malaria.

Sensitivities of immunologic methods for detecting malaria remain a problem. In non-endemic areas for malaria, the parasitemia is often very lower than the detection capability of devices involving capture of HRP-2 and pLDH and is highly expensive [8].

Complete Blood Count (CBC) is the most commonly done laboratory test asked for almost

Table 6. Summary of studies evaluating malaria diagnosis accuracy with automation

Author	Year	Instrument/ Test	Sensitivity (%)	Specificity (%)	Malaria factor
Mendelow et al. [18]	1999	Depolarized Laser Light	72	96	
Hanscheid et al. [22]	2001	CD3500, Abott	95	88	
Grobusch et al. [5]	2003	CD3000, Abott	48.6	96.2	
Scott et al. [19]	2003	CD4000, Abott	80.2	87.3	
Yoo HJ et al. [20]	2010	Sysmex XE-2100	46.2	99.7	
Fourcade et al. [9]	2004	Beckman Coulter	96.8	82.5	5.1
Briggs et al. [8]	2006	Beckman Coulter	98	94	3.7
Kang et al. [16]	2008	Beckman Coulter	81.8	72.3	4.57
Indira et al. [21]	2015	Beckman Coulter	97	89	3.4
Present Study	2011	Beckman Coulter	92.5	83.7	2.7

all the febrile illnesses that might include malaria patients. It is available extensively in developing as well as developed countries. Even though the first automated analyser was built in 1953, it was not until 1990s when alterations in CBC was first noted and reported [6,15]. Ever since, many studies have been conducted in malaria diagnosis with automation using Cell-Dyn (3500 and 4000), Coulter (GEN.S and LH) and Sysmex analysers. Results varied greatly in sensitivity and specificity from 48 to 100%, best being with studies involving Cell Dyn 4000 series and coulter [5,6,8,9,16,17]. We evaluated the feasibility of LH750 Beckman Coulter as a rapid and cost effective method to detect Malaria especially in cases, which lacks clinical suspicion.

In our study, we concluded that SD in lymphocyte and monocyte volumes are most accurate in malaria detection, which was first reported in 2004 by Fourcade et al. [9]. He was the first to include the possibility of using a discriminant factor to raise a suspicion flag in the analyser. In his study, he used the same parameters and used a cut off value of 5.1 to obtain 96.9% sensitivity and 82.5% specificity. Subsequent study published in 2006 by Briggs et al [8] had a better sensitivity and specificity (98% and 94%) using a different cut off value of 3.7. The malaria factor used for *P. falciparum*, *P. vivax* and *P. ovale* was 6.2, 5.9 and 5.7 respectively. Both studies showing malaria factor is not a fixed number and has to be standardised to the machine and to the population who is being evaluated with that. We analysed three groups with malaria positive, malaria negative with fever and healthy individuals. By using logistic regression the malaria factor derived was using SD volume of monocytes and lymphocytes. Cut-off of 2.7 as Malaria factor yielded specificity of 92.5% and sensitivity of 83.7%.

The findings from present study is in concordance with most of the previous studies except two studies [5,6] which reported low sensitivity. Combining Malaria factor with other complimentary parameters like variation in monocyte volume and platelet count reduction could increase the detection rate, which needs further studies. Table 6 shows studies with their sensitivity and specificity from literature [18–22] regarding the use of Automated Haematology Instruments in Malaria detection.

The present study demonstrated a fully automated rapid method for the potential detection of malaria infection in adults. However, as already seen the malaria factor is not a constant factor and needs to be standardised for the population. Advantage of this being results of the malaria factor will be available at the same time as part of the CBC count and at no extra cost because these data are reported automatically with the differential count results. Hence, this can be implied as an effective method to raise a malaria suspect flag especially among the malaria endemic area after standardisation.

5. CONCLUSION

By using a previously described algorithm, a “suspect malaria” flag could be generated on the analyser, allowing the detection of cases of unsuspected malaria and therefore, early diagnosis of the disease.

We cannot consider the suspect malaria flag as gold standard diagnostic method. It should be used as an indicator to screen the samples for malaria by specific methods when reported the flag. Most of the studies on this modality of malaria detection being done in non-endemic area and India being an endemic area demands

more study towards automated detection of Malaria. This study involves a small sample size from a specific area and hence, the positive findings in the specific population cannot be extrapolated.

6. LIMITATIONS

Further studies with large sample size and comparison with RPA technique may aid in development of a fully automated, rapid, more sensitive and specific method for malaria detection. Standard values in malaria factor are still unknown and varies with study and population. Further prospective study is required to assess the detection rate with the described malaria factor to establish its validity. Confounding factors like age and gender is not being addressed in the study which if included as variables can yield a more specific factor with better detection rate.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Institute Ethical Committee had approved the study IEC231/2011.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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