Case Report

Successful detection and species differentiation of malarial parasite using an automated hematology analyser: A case series

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Abstract

Malaria is a global burden and requires accurate and early diagnosis. WHO has recommended that all clinically suspected cases should have parasitological confirmation for definitive treatment. There are other many methods which are expensive and require high skills. Hence, incorporation of reliable methods into automated hematology analysers will help identify malarial parasites at the earliest. Here we present three cases in which Plasmodium were identified on autoanalyser.

Keywords: Malaria, Automated, Hematology analyser, Plasmodium, scatterplot

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Introduction

Malaria continues to be an important parasitic infestation worldwide affecting 3.2 billion people globally. To overcome this burden of malaria efficiently, accurate and early diagnosis is pivotal. Clinical diagnosis has low specificity because the symptoms and signs are non-specific and are similar to many other tropical infections. The world health organization (WHO) has recommended that all clinically suspected cases of malaria should have a parasitological confirmation to avoid presumptive treatment and to curtail unwarranted use of anti- malarial drugs [1].

The two most commonly used tests for demonstration of parasite are peripheral smear examination and immunochromatographic pan- Plasmodium LDH antigen detection based rapid diagnostic test (RDT) [2]. It is worth mentioning that, the other modalities like polymerase chain reaction (PCR), loop-mediated isothermal amplification (LAMP) and flow cytometry based parasite DNA or RNA detection are highly sensitive and specific in malarial detection. But these methods are expensive, laborious, non-automated and require sophisticated skills [1]. In order to overcome this, incorporation of a reliable method into automated hematology analysers will help to identify

malarial parasites in blood sample early and helps in initiation of treatment judiciously. Here we present three cases of malaria detected on HORIBA YUMIZEN H550 automated analyser (HORIBA ABX SAS, YUMIZEN H550, 2017) and we are also presenting a mini- review on the detection of Plasmodium by automated analysers.

Case series:

We report three cases of malaria which were detected on automated hematology analyser during routine complete blood count (CBC) work up. Two cases had history of fever and one was afebrile. The case details are as follows

Case 1:

A 35-year-old man presented with abdominal pain, nausea with history of 2 units blood transfusion for the treatment of anemia. Patient had no history of fever or vomiting. On examination patient was anxious, afebrile with blood pressure of 102/74 mm Hg and pulse rate of 120/min. Abdomen examination revealed tender hepatosplenomegaly which was later confirmed by Ultrasound examination.

Routine CBC test revealed low hemoglobin level with normal white cell counts & platelets with a flagging for malarial parasite? Plasmodium falciparum. The scoring given by the analyzer: Plasmodium falciparum: 0.51; Plasmodium vivax: 0.1; Dengue: 0.1. Blood smear examination subsequently done was positive for Plasmodium falciparum with many gametocyte forms. (Figure 2). Detailed blood counts, analyzer scoring & the scatterplot generated using HORIBA YUMIZEN H550 automated analyzer are shown in Table 1, Table 2 & Figure 1 respectively.

Case 2:

A 70-year old elderly male presented to OPD with complaints of difficulty in breathing, fever with chills, cough, rhinorrhea and myalgia. On examination oxygen saturation was 97%, temperature 97°F, pulse rate 125/min. Dengue serology with CBC & smear for malarial parasite were sought. Dengue serology was negative.

CBC test showed moderate thrombocytopenia with normal hemoglobin & white cell counts. Scatterplot generated using HORIBA YUMIZEN H550 automated analyzer displayed a clear cut grey area in the left bottom corner of the scattergram (Figure 1b) with flagging for malarial parasite ? Plasmodium vivax. The scoring given by the analyzer: Plasmodium falciparum: 0.1; Plasmodium vivax: 0.95; Dengue: 0.27. Blood smear examination was also positive for Plasmodium vivax (Figure 2) with many trophozoites, schizonts & occasional gametocytes. The blood counts, analyzer scoring & the scatterplot are shown in Table :1, Table 2 & Figure 1 respectively.

Case 3:

We received EDTA sample of a 17-year old male with history of fever. CBC test revealed moderate thrombocytopenia with normal hemoglobin & white cell counts. Scatterplot generated using HORIBA YUMIZEN H550 automated analyzer showed a clear cut grey area in the left bottom corner of the scattergram (Figure 1b) with flagging for malarial parasite ? Plasmodium falciparum. The scoring given by the analyzer: Plasmodium falciparum: 0.5; Plasmodium vivax: 0.2; Dengue: 0.35. Blood smear was also positive for Plasmodium falciparum. The blood counts, analyzer scoring and the scatterplot are shown in Table: 1, Table 2 & Figure 1 respectively.

Cases summary: Cases 2 and 3 both presented with fever and case 2 in addition had difficulty in breathing. CBC done for both showed normal haemoglobin & white cell count but with low platelet counts. Case 1 didn't give history of fever and on evaluation found to have anemia. Malaria infection produces a wide variety of

symptoms, ranging from absent or very mild symptoms to severe disease and even death. The manifestations of severe disease includes anemia, respiratory distress, acute kidney injury and coma. All of our cases had severe manifestations of malaria, one cases had anemia, one had thrombocytopenia, and the other had thrombocytopenia with dyspnoea.

Blood smear examination of the cases (Figure 2) revealed Plasmodium falciparum in 2 of the cases and vivax in one other case. Study done by Hanson et al [8] in severe cases of Plamodium falciparum reported that the admission platelet count was inversely related to parasitemia, amount of microvascular sequestration and disease severity which is in correlation with the study by Das et al [9] who stated that patients with severe /complicated malaria had significantly low platelet counts compared to uncomplicated malaria cases. Similar to their study out of 3 cases in our study 2 cases had thrombocytopenia, one was infected by Plasmodium falciparum and the other by vivax. However the severity of thrombocytopenia is more in patient with plasmodium falciparum infection, who also had high blood parasitemia.

Table 1: Hematological profile in our cases

Parameters	Case 1	Case 2	Case 3
RBC Count (10^6/µl)	2.18	4.25	4.02
Hemoglobin (g/dl)	6.8	12.7	12.5
Hematocrit (%)	19.4	37.3	36.2
Platelet Count (10^3/µl)	155	43	22
Total WBC Count(10^3/µl)	6.8	5.02	3.96
Differential Count (%)			
Neutrophils	63	60	69.2
Lymphocytes	29	24	17
Monocytes	7	16	13
Eosinophils	1	0	0.8
Basophils	0	0	0

With regard to the CBC results generated by Automated analyzer HORIBA YUMIZEN H550, suspected pathologies including malaria suspicion message along with malaria & dengue scoring were shown up in the result screen for all three cases. The Malaria and Dengue suspicion messages are triggered in the CBC display when one of these scores is higher than the limit set up in the software (Malaria P. falciparum? Score > 0.50, Malaria P. vivax? Score > 0.31, Dengue? Score > 0.50) [3]. All of our cases had reached or gone beyond the limit set up and thus appears more promising in detection and species identification of malarial parasites. The limit set up value, the values reached in each case along with the species identified by the analyzer are shown in the table below Table 2.

Table 2: Score set in HORIBA YUMIZEN H550 and score in our cases

Infection	Limit Set Value	Case 1	Case 2	Case 3
		P.falciparum	P.vivax	P.falciparum
Plasmodium falciparum	0.5	**0.51**	0.1	**0.5**
Plasmodium vivax	0.31	0.1	0.95	0.2
Dengue	0.5	0.1	0.27	0.35
P.falciparum - Plasmodium falciparum; P.vivax – Plasmodium vivax				

The purpose of the infectious suspicion messages & malaria dengue scoring is to provide a screening flag for triggering out suspected Malaria and Dengue infections and is only for intended use in a clinical laboratory and not for patient diagnosis. Hence requires confirmation by using reference method. Here in our study cases, blood smear examination confirmed the suspicions and hence the flagging & species scoring appears to be sensitive in detection of malaria & its species. The sensitivity & specificity of infectious screening messages assessed through onsite validation studies [3] are shown in Table 3.

The scatter matrix generated by the analyser for all the three cases (Figure 1) have shown a separate grey cluster in the left corner of the scattermatrix with lymphocyte interference. This alarm indicates the possible presence of Platelet aggregates, Small lymphocytes, Erythrocyte membrane resistant to lysis (stroma), Erythroblasts (NRBC), Infected erythrocytes. By blood smear examination the reason for the interference in all our cases was found to be parasitized RBCs.

Table 3: Sensitivity and specificity of HORIBA YUMIZEN H550

Species	Sensitivity	Specificity
Plasmodium falciparum	48%	92%
Plasmodium vivax	72%	98%
Dengue	79%	72%

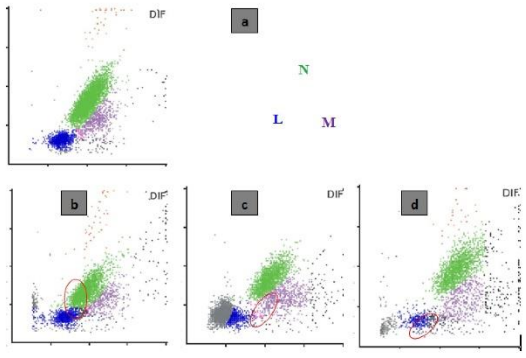

Figure - 1: The volume/scatter matrix generated using HORIBA YUMIZEN H550 automated analyzer and the representative control normal scatterplot pattern is shown. a) Representative normal scatterplot with no lymphocyte interference. L- Lymphocyte M- Monocyte N- Neutrophil; b-d) The grey area marked in red circles in left most corner of WBC matrix represent the infected RBCs with malarial forms.

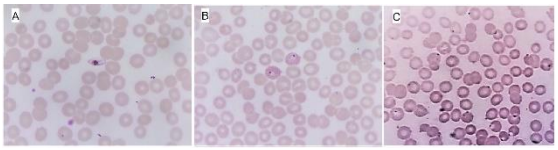

Figure - 2: Giemsa Stained thin blood smears (10 x 100 magnification) a) Banana shaped gametocytes of Plasmodium falciparum b) Trophozoites of Plasmodium vivax c) Multiple RBCs infected by Ring stages of Plasmodium falciparum

Discussion

Malaria is a global disease and approximately 229 milion cases were reported in 2019 worldwide [4]. Mortality rate due to malaria globally ranges between 0.3 and 2.2% and in regions with severe forms it is between 11-30%. The public health impact of malaria is variable depending on the parasite species and its transmissibility [5]. The clinical features of malaria is non-specific and needs parasitological confirmation for its diagnosis. Microscopy has been the gold standard method over a century for routine diagnosis. It allows species identification and determination of parasitemia with a detection threshold of 4- 100 parasites/µl. But laboratory misdiagnosis is not unknown based on microscopy because of lack of expert microscopists, periodic training and lack of equipment. In order to overcome this lacuna, alternate diagnostic methods like immunochromatographic rapid diagnostic test (RDT), polymerase chain reaction, Loop Mediated Isothermal Amplification (LAMP), quantitative buffy coat (QBC) and flow cytometry based parasite DNA or RNA detection were developed [1]. But these tests are time-consuming, expensive and non-automated. Thus, incorporating a reliable method for identifying malarial parasite by the automated hematology analysers was the need of the hour for early diagnosis and to reduce the adverse effects of malaria worldwide.

The automated hematology analysers work on fundamental principle of flow cytometry by VCS (volume, conductivity, scattering) technology and produce forward and side scattergrams. This robust technology has been found to be suitable to detect low

concentration of parasite in blood samples. Many studies have reported the efficacy of automated hematology analyser in diagnosis of malaria and Cell- Dyn 3500 was the first analyser to detect malaria. The hemazoin pigment liberated in malaria are birefringent and are engulfed by neutrophils, monocytes and macrophages. Hemazoin pigment because of birefringent property is able to scatter laser light and the analyser detects the parasites depending on their size and pigment.Thus, results in abnormal scattergrams in WBC region.This principle was adopted in Cell-Dyn and Beckman Coulter. But the drawback of this principle is that detection of malaria was dependent on total parasite burden and the quantity of hemozoin containing cells.Thus, this method lacks sensitivity for early infection. The severity and chronicity of malaria is directly related to hemazoin load and quantification of hemazoin laden macrophages and granulocytes by automated cell counters serve as surrogate disease severity laboratory marker [6].

Automated analysers not only detect malaria but also identifies various forms of stages of parasite. This was seen to be achieved by using a cell lysis detergent and nucleic acid fluorescence tagging using Sysmex SIF cytometer. The cytoskeleton of infected red blood cells (iRBCs) is remodeled by Plasmodium resulting in alterations in membrane properties of iRBC. This results in resistance to lysis for iRBCs those harboring mature parasite forms. These unlysed iRBCs produce false signals giving a peak on left of WBC volume histogram or as separate cluster. This method has been adopted by Sysmex XE, XN series and Mindray. Since cytoskeleton remodeling is greatly influenced by mature forms of Plasmodium, this technique lacks sensitivity for early infection and for those with low parasitic index [2] .
As a recent development Sysmex XN-30 analyser has developed a dedicated module for detection of Plasmodium by embedding a 405 nm violet laser with scattering and fluorescence measurements. This model allows detection and counting of Plasmodium using partial lysis reagent allowing parasites to remain inside iRBCs and nucleic acid staining for labeling parasites DNA. It has the advantage of improved detection limit of 20 parasites/ µl. It also identified gametocytes as a separate cluster on scattergrams [7].

Yumizen H550 used in the CBC analysis of our cases works with the technology of impedance for WBC counting and for differentials. WBC channel provides a volume/scattering matrix for the 5 differentials. WBC DIFF Alarms are triggered in the analyzer whenever there is lymphocyte, monocyte interference or abnormal differentiation of leucocytes.The scattermatrix generated by the analyser for all the three cases in our study (Figure 1) have shown a separate grey cluster in the left corner of the scattermatrix with lymphocyte interference. This alarm indicates the possible presence of Platelet aggregates, Small lymphocytes, Erythrocyte membrane resistant to lysis (stroma), Erythroblasts (NRBC), Infected erythrocytes [3]. The reason for the interference in all our cases was found to be parasitized RBCs. In one of our malaria cases separate tight cluster due to mature forms of Plasmodium vivax seen (Figure 1c). These mature forms have remodeled the cytoskeleton of iRBC which results in increased lyse resistance. These mature forms are large enough to be detected by the optical plus impedance technology [2] . In addition to the alarms, when the Malaria mode is activated in the analyzer, the Malaria and Dengue scores are calculated and displayed on the Results screen. The Malaria and Dengue suspicion messages will pop up when one of these scores is higher than the limit set up in the software.

Our cases have reached or gone beyond the limit set up value (Table 3) and hence appears promising in detection of malaria and its species.

Automation of malaria is now possible with most of the available standard automated cell counters. Detection of malaria as a complement to its main purpose of CBC analysis is serving as ancomplementary diagnostic tool in investigating pyrexia of unknown origin. This will help in early diagnosis of malaria and reduces the adverse outcomes resulting from delay. Asymptomatic carriers can be identified efficiently and appropriate treatment will reduce the burden of the disease in long run.

Conclusion

Automated blood cell analysers are helpful in timely diagnosis of clinically unsuspected cases of malaria and thus prevent adverse clinical outcomes. Hence, detection of malaria as a by-product of hematology analysers serve as an complementary diagnostic tool in febrile patients. Modern CBC analysers help technicians and pathologists to screen for malarial parasite and to confirm by peripheral smear at low cost and thus reducing false negatives.

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Abbreviations

WHO - World health organization
RDT - Rapid diagnostic test
PCR - Polymerase chain reaction
LAMP - Loop-mediated isothermal amplification
RDT - Rapid diagnostic test
LAMP - Loop Mediated Isothermal Amplification
QBC - Quantitative buffy coat

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