Case Report

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Incidental detection of malaria parasite in automated hematology 6-Differential analyzer

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Abstract:

India has been facing the problem of malaria for centuries. Peripheral smear microscopy remains the gold standard for diagnosis; however, it is a tedious process and requires qualified staff. Flow cytometric-based hematology analyzers' scattergrams can be of vital use in identifying the abnormal scattergrams for malaria. We report a patient with fever and chills who presented to the outpatient department and was further evaluated for pyrexia of unknown origin. 6-Diff complete blood count analyzer incidentally showed abnormal scattergram which was evaluated by a pathologist and confirmed the presence of *Plasmodium vivax*. The accuracy in detection of malaria diagnosis can vary based on species, parasite load, immunity, and clinical context. Pathologist and technical staff should analyze any abnormal scattergram and hematological data along with peripheral smear to identify the parasites.

Keywords:

6-Diff complete blood counter, flag, malaria, peripheral smear, scattergram

Introduction

Microscopy-based diagnosis is performed for the identification of malaria parasites with a parasitemia threshold of 4–100 parasites/μL. However, in countries like India, laboratory misdiagnosis is not uncommon so is the case with developed countries where imported malaria can be missed by laboratories who very rarely deal with such cases.[1,2]

Complete blood count (CBC) is one of the most frequently requested laboratory tests indicated for various conditions, along with febrile patients. The hematology analyzers work on the principle of flow cytometry by volume, conductivity, and scattering technology with scattergrams. The hemozoin pigment formed in malaria infection can cause scattering of light and producing various abnormal scattergrams.[1,3] The incorporation of the malaria flag in analyzers will allow for early detection of malaria disease.[4]

Case Report

We report a case of unexpected detection of malaria in routine CBC run of a male patient aged 42 years, who had visited our outpatient department with complaints of fever and chills for 3 days. The patient was a native of Kolkata, West Bengal, which is an endemic zone for malaria in India; he had recently traveled to Southern India. He had no previous history of malaria infection. Physical examination of the patient revealed elevated body temperature of 38°C with chills and blood pressure 120/60 mm of Hg. He was advised to undergo routine laboratory investigations. The laboratory results were as follows: white blood cell (WBC) count -8 × 10^3/μL, differential leukocyte count (neutrophils and precursor - 51%, lymphocytes - 42%, monocytes - 5%, eosinophils - 2%, and basophil - 0%), hemoglobin - 13 g/dL, hematocrit- 38.9%, total red blood cell count -

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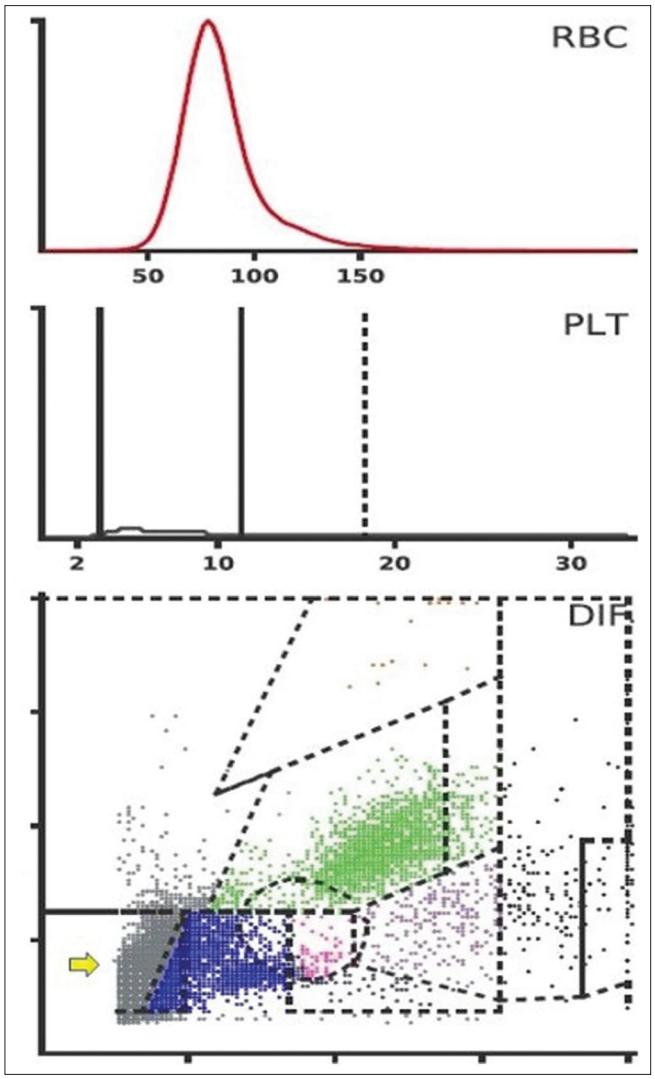

Figure 1: Histogram and scattergram from 6-Diff Horiba Yumizen H550 showing the gray-colored area (yellow arrow) of white blood cell with background noise, lymphocyte interference

4.47 × 10^3/μL, mean cell volume - 87.0 fL, mean corpuscular hemoglobin - 30.8 pg, mean cell hemoglobin concentration - 35.4 g/dL, and platelet (PLT) count - 20 × 10^3/μL. His NS1 antigen and immunoglobulin M testing for dengue were negative.

The patient routine urine and microscopy were found to be normal. The malaria rapid antigen card test was negative in our patient. We had performed CBC analysis using automated hematology analyzer 6-diff Horiba Yumizen H550 [Figure 1], and the flags which were displayed on the machine were WBC (background noise, lymphocyte interference), PLT (RBC–PLT interference), and suspected pathologies (thrombocytopenia, PLT aggregate, or nucleated red blood cells, large immature cells). The WBC scattergram was showing gray-colored ghost cells on the left to lymphocytes.

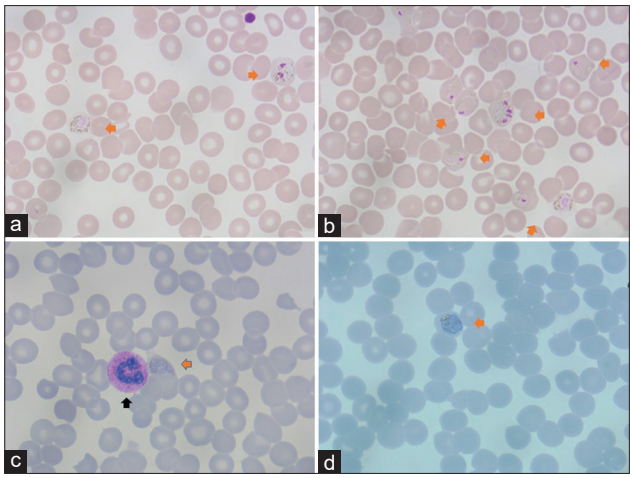

Figure 2: (a-d) Peripheral blood smear microscopy showing enlarged red blood cells with trophozoites of *Plasmodium vivax* having fragmented nuclei and golden brownish pigment within the enlarged amoeboid red blood cells (orange arrow) along with band forms (black arrow) (Leishman stain, ×100)

Based on warning flags, we prepared peripheral blood smear and stained them with Leishman stain, and our pathologist examined peripheral blood smear microscopy which revealed trophozoites of *Plasmodium vivax* with fragmented nuclei and golden brownish pigment seen in the enlarged amoeboid RBCs and parasite index of 8% [Figure 2]. Few macrocytic RBCs were also seen on the peripheral blood smear. The leukocytic differential showed a mild shift to left with few metamyelocytes and band forms [Figure 2]. Manual count of PLTs count revealed a reduced value (20 × 10^3/μL) with few giant PLTs.

Discussion

Malaria diagnosis depends on clinical observations and diagnostic tests (microscopic detection, quantitative buffy coat method, and rapid card tests). These methods have their drawbacks and limitations.[5]

The rapid card test for malarial parasites can be negative with low parasite index (<100 parasite/μL) or only when the viable parasite produces the parasite lactate dehydrogenase.[6,7] Peripheral smear examination is considered the gold standard for reporting of malaria with proper training of staff in microscopy.[8] Current generation 6-diff CBC analyzers with abnormal WBC scattergrams can be a helpful aid for the technicians and pathologists suspect for malaria and use the peripheral smear technique for confirmation.[9]

Hemozoin, a brown crystalline pigment when produced during hemoglobin catabolism, is detoxified by malarial parasites. These pigments are then phagocytosed by neutrophils, monocytes, and lymphocytes allowing the identification of the cells as abnormal scattergrams.[8-10]

Thrombocytopenia is a very common association with malaria. It becomes a more important factor in febrile patients, travelers from endemic places. Studies have shown a strong association of thrombocytopenia with *P. vivax* in the Indian subcontinent so was in our case.[11,12]

As per company standards, CBC analyzers such as Cell Dyn 4000 (Abbott Diagnosis, Sysmex XE-2100(Coulter Gen), LH750 (Beckman Coulter counter), and Horiba Yumizen H550 can show flag warning for parasitic infection.[4,6,8,9,13] Technician trained to identify the flags from CBC analyzers can help in the timely screening of malaria infection.

Conclusion

This report provides us clues in looking for abnormal CBC scattergrams and peripheral blood smear examination being the gold standard reliable technique for the detection of the malarial parasite. Current generation automated hematology analyzers can be programmed to identify infected RBC (gray color in Horiba Yumizen H550) for the detection of malarial parasite infection.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient (s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initial s will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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