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MALARIA DIAGNOSIS BY ABNORMAL SCATTERGRAMS IN AUTOMATED HEMATOLOGY ANALYZER

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ABSTRACT

Background: *Malaria remains one of the important infections that have caused a great social, economic, and medical impact, more so in underdeveloped countries.* ***Aims:*** *The aim of this study was to compare the efficacy of hematology analyzer SysmexXE-2100 with that of conventional methods for diagnosing malaria. Material and* ***Methods:*** *A prospective study was carried out in our teaching hospital from July to December 2015. Febrile patients of all ages and both sexes with a provisional clinical diagnosis of malaria were included in the study (n=5,607). Two milliliters of blood was collected in an EDTA vial. The samples were subjected to Complete Blood Count (CBC) by analyzer and malaria detection by Giemsa stained thick and thin films and Quantitative Buffy Coat (QBC) method.* ***Results:*** *A total of 5,607patients were clinically diagnosed as malaria cases and 201among they were found positive for malaria by Giemsa stained film and 256 by QBC method. Abnormal scattergrams were shown by the analyzer in 179 out of 256 malaria positive cases. The commonest pattern was extended neutrophil zone with less space between neutrophil and eosinophil (n= 97), followed by double neutrophil (n=-39). An abnormal WBC/BASO plot was observed in 42 malaria patients. The overall sensitivity and specificity of the analyzer was found to be 76.88% and 67.86% respectively.* ***Conclusion:*** *Hematology analyzers are very useful in timely diagnosis of malaria; by flagging abnormal events in the plot give a clue towards diagnosing malaria. Particularly in the absence of a clinical request, diagnosis of malaria may not be missed.*

KEY WORDS

Malaria , automated analyzer, scattergram

INTRODUCTION

Malaria is a common parasitic disease and a major public health problem worldwide and a major cause of death in tropical and sub-tropical countries; 90% of fatalities occur in children of underdeveloped nations. Although effective ways to manage malaria now exist, the number of malaria cases is still increasing, due to several factors. For more than a century, the diagnostic approach to malaria is by clinical features and microscopy. As malaria can present in varied clinical forms, clinical diagnosis alone is unreliable and should be confirmed by laboratory tests. The microscopic methods of detection of Plasmodia are the standard method of malaria diagnosis till date. Though this is a cost effective method, there is a high degree of subjectivity involved in reporting of stained smears. Correct interpretation of the film requires considerable expertise. The microscopic method becomes still less reliable in cases with low degree of parasitemia. Hence laboratory misdiagnosis is not uncommon.

Due to lack of trained personnel, and the need for training, several alternate methods have been designed. Rapid diagnostic tests by immunochromatography have become very popular. But these special tests are requested only on strong clinical suspicion of malaria. Complete Blood Count (CBC) is one of the commonest laboratory tests that are requested for almost all the febrile cases. CBC is also done for febrile patients who are clinically misdiagnosed as fever other than malaria. Currently CBC test is performed using automated hematology analyzers in most of

the hospitals. The hematology analyzers work on the principle of flow cytometry in which flow cells scatter a beam of laser light focused on them producing scattergrams. [1]

Sysmex XE-2100, an automated analyzer uses a semiconductor laser to give optical information about the cells. The normal scattergram in the DIFF plot comprises five components: lymphocytes(pink), monocytes(green), neutrophil and basophils (blue), eosinophils (red) and a space between the neutrophil and eosinophil populations.[2]Hemazoin pigments produced in malaria also scatter the laser light, thus exhibiting several abnormal scattergrams during routine CBC analysis.[3]While analyzing a CBC scattergram, an event in the plot points towards probably diagnosing malaria. Hence this study was designed with an aim to compare the efficacy of Sysmex XE-2100 hematology analyzer with that of conventional methods for diagnosing malaria.

MATERIAL AND METHODS

A prospective study was carried out at the Central Diagnostic Laboratory of our teaching hospital from July to December 2015. Febrile patients of all ages and both sexes with a clinical suspected of malaria were included in the study (n=5,607). Informed consent was obtained from patients or parents as appropriate.

Two milliliters of blood was collected in an EDTA vial. The samples were subjected to CBC by analyzer and malaria detection by conventional techniques, i.e. Giemsa stained thick and thin films and Quantitative Buffy Coat (QBC) method.

Thick and thin blood smears were prepared as per the standard method. The smears were stained with Giemsa stain. Approximately 100 fields were examined over 10 minutes by an experienced microscopist.

In the QBC technique, approximately 50-60µl of blood was taken into a capillary tube coated with acridine orange, potassium oxalate and fitted with a cap. The tubes were spun in the QBC microhaematocrit centrifuge at 12000 rpm for 5 minutes after inserting a plastic float inside the tubes. The tube was then mounted on a plastic holder and examined through a light microscope with paralens attachment. Approximately 20 fields were examined over 2-4 minutes.

For the purpose of this study, a positive case of malaria was defined as positive by either Giemsa and/or QBC method. The cases were followed up and repeat sampling done for the same tests, after 72 hours of initiation of antimalarial agents.

The scatter grams in the DIFF and WBC/BASO plot of the analyzer were analyzed. The findings of microscopy were compared and matched with the abnormal scattergrams produced by the analyzer. The initial sample findings were also correlated with the findings in the follow up samples.

RESULTS

A total of 5,607 febrile patients were enrolled for the study and 201 among them were found positive for the Plasmodium species by Giemsa stained film and 256 by QBC method. All the cases found positive by Giemsa stained film microscopy were also positive by QBC method.

Plasmodium vivax was the most predominant species (n=211), followed by P, falciparum (n=31) and 14 had mixed infection with both these species. Abnormal scattergrams were shown by the analyzer in 179 out of 256 malaria positive cases. The various patterns in DIFF scattergram included double neutrophil, extended netrophil with decreased space between eosinophil and neutrophil, double eosinophil, grey zone and a combination of these. The commonest pattern was extended neutrophil zone with less space between neutrophil and eosinophil (n=97), followed by double neutrophils (n=39) (Table 1)

An abnormal WBC/BASO plot was observed in 42 malaria positive patients. The overall sensitivity and specificity of the analyzer was found to be 76.88% and 67.86% respectively.

Table 1: Patterns of scattergrams.

Type of abnormal scattergram	Number (N= 179)	%
Extebded neutrophil zone	97	54.2
Double neutrophil	39	21.8
Abnormal WBC/BASO plot	42	23.5
Combination patterns	21	11.7

DISCUSSION

Giemsa stained film remains the gold standard test to diagnose malaria.[4] In well trained microscopist's hands, this method is expected to detect 50 parasites/ microlitre(0.001%) parasitemia.[5]Hence lack of expertise may lead to misdiagnosis, thus increasing malaria associated with complications.

QBC method is used in many laboratories as the initial screening technique and in some as a backup for microscopy. [6] As the acridine orange dye is a non-specific stain, the Howell- Jolly bodies also take up the stain. Parasites other than P. falciparum are often hidden in the mononuclear layer of higher density and hence may get missed under the fluorescent light making QBC method prejudicial.[7]

CBC is a very common investigation performed on blood samples of febrile patients. Diagnosing malaria during a routine CBC test could definitely help detect the cases early and potentially reduce the adverse outcomes of malaria. Routine CBC analysis is performed on automated analyzers and there is a growing interest in malaria detection using these analyzers.[8]

The Sysmex XE-2100 is a fully automated five part differential hematology analyzer. The cell counter uses combined impedance and radiofrequency conductance detection. The semiconductor red-diode laser light passing through the blood sample is scattered in three different aspects to find different leucocyte population. At the end of each run, 32 clinical variables and 7 scatter plots are obtained.

The hemazoin pigments are produced by the breakdown products of RBCs in malaria. The monocytes, the macrophages and the neutrophils engulf these pigments. The hemazoin pigments are birefringent in nature and are capable of scattering laser light, hence showing abnormal scattergrams. Studies from the west have reported the usefulness of analyzers in infections like malaria and dengue.[9]A study from North India, reported a correlation between abnormal scattergrams produced by flowcytometric analyzer with various forms of malaria parasite found during the microscopic examination.[10].

The flowcytometric analyzer identifies the parasites and depending on their size, nuclear and pigment content they appear in the DIFF plot in the area of neutrophil and eosinophil. Eosinophils are more granular and have less nuclear material compared to the neutrophils. Hence, in the normal DIFF plot (Fig 1), they are placed to the right of neutrophils.

The atypical neutrophil patterns (i.e. extended neutrophil, reduced space between neutrophil and eosinophil and double neutrophil) may be caused by malarial parasites of various stages. Our finding also suggested that blood samples with a predominant form of gametocyte appear as the extended neutrophil zone with less space between neutrophil and eosinophil andcorrelated with finding gametocyte stage by peripheral smear. Depending on the stage of gametocyte (mature/immature), schizoint, and late trophozoite present in the blood sample, double neutrophil like picture may be observed (Fig 2).

A significant correlation has been reported between the number of late trophozoites, schizonts, and gametocyte of *P. vivax* and abnormalities formed in the DIFF, WBC/BASO, and RET-EXT scattergrams. [11] Though neutrophils form only the second line of defense in malaria infection, and the turnover of pigment containing WBC varies individually, neutrophil-containing pigment is observed under heavy parasitaemic condition and considered as a poor prognostic indicator. [12],[13].[14],[15] The blue coded event in the WBC/BASO plot is due to RBCs infected by

Plasmodia that appear in the RBC ghost area. As in P. vivax infection, all forms of parasite are present in the peripheral blood; there is a prominent blue coloured event in vivax malaria compared to falciparum malaria.

Several authors have suggested that spurious eosinophilia and double eosinophil graphs in analyzer findings of malaria cases are abnormal representation of pigment-containing neutrophils. [16] We found, double eosinophil population in the DIFF plot in malaria blood samples with a predominant stage of trophozoite. The follow up samples after 72 hours of initiation of antimalarial treatment showed that, the WBC/BASO plot reverted to normal earlier than the DIFF plot.

Fig 1: Differential scattergram in normal blood.

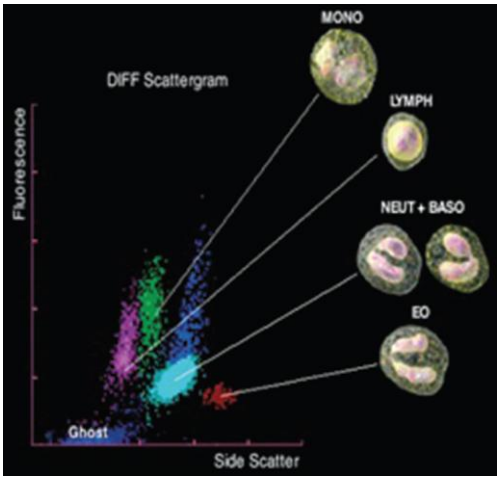
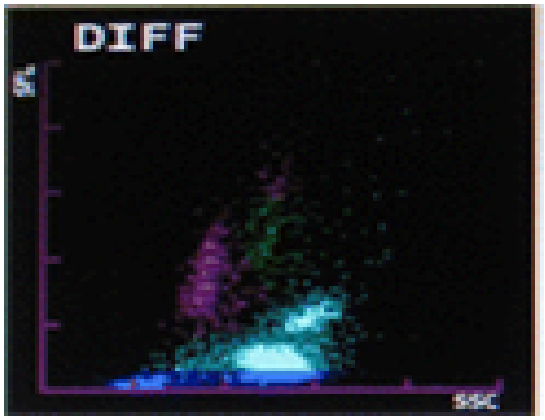


Fig 2: Differential scattergram showing double neutrophil in malaria case.



CONCLUSION

Fever is a very common clinical presentation of many infectious and non-infectious conditions. Differentiating its causes at the onset is challenging. The management of the patient depends on the etiology, and therefore it is important to find the origin of fever. Hematology analyzers are very useful in timely diagnosis of malaria; by flagging abnormal events in different areas of the plot give a clue towards diagnosing malaria. Awareness regarding such specific patterns, is helpful particularly in the absence of a clinical request, diagnosis of malaria may not be missed. Hematologists should review the analyzer data everyday to acquaint with plots so as pick up unsuspected cases. However, such cases have to be confirmed by a gold standard method for malaria diagnosis.

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