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THE IMPORTANCE OF AUTOMATED HEMATOLOGY ANALYZER WITH MULTI ANGLED POLARIZED SCATTER SEPARATION (MAPSS) BASED FLOW CYTOMETRY

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ABSTARACT

Objective: To evaluate the efficacy of Multi Angled Polarized Scatter Separation (MAPSS) based flow cytometric principles for detection of malaria by detecting malarial pigment hemozoin.

Materials and Methods: This study was carried out North-West General Hospital and Research Centre Peshawar from July 2011 to Sepstember 2011. A toal of 10032 patients were included in the study. Complete blood counts were done on automated hematology analyzer, Abbott CELL-DYN RUBY. Comparison Multi Angled Polarized Scatter Separation (MAPSS) based flow cytometry detection of hemozoin containing leukocytes was done with thin and thick film microscopy of giemsa stained smear.

Results: Total of 144 (1.43 %) cases of malaria were diagnosed on microscopy. 132 cases were vivax species (91.66%) and remaining 12 cases (8.3 %) were falciparum species. The Multi Angled Polarized Scatter Separation (MAPSS) flagged 104 cases. (72.2 %) , of which 40 cases could be typed as 38 (95 %) of plasmodium vivax and 2cases (5%) plasmodium falciparum.

Conclusion: The introduction of automated hematology analyzer having Multi Angled Polarized Scatter Separation (MAPSS) based flow cytometric principle for detection of malarial pigment hemozoin is of great importance in malaria endemic countries. This reduces the chances of missing diagnoses and in clinically unsuspected cases where microscopy for malaria parasite has not been advised.. It also reduces time of cumbersome microscopy when malaria is not flagged.

Key Words: Multi Angled Polarized Scatter Separation, Malaria, Plasmodium, Vivax, Falciparum.

INTRODUCTION

Malaria is known to cause several changes in full blood count (FBC) parameters, of which the most prominent are anaemia and thrombocytopaenia. The time of taking the blood sample, slide preparation and staining can affect the quality of microscopic diagnosis and requires considerable technical expertise[1]. Diagnosis of malaria is usually made by microscopy [Giemsa, Acridine Orange (AO), and Quantitative Buffy Coat (QBC) assay], which requires expertise. Currently, automated haematology analyzers are being used for complete blood count (CBC), in all acute febrile and non-febrile illnesses which simultaneously detects malaria.

The detection of malaria pigment, hemozoin (Hz) containing leukocyte in the circulation is an indication of the presence of plasmodium species. The Multi Angled Polarized Scatter Separation (MAPSS) based flow cytometry used in automated instruments also detect the Hz containing pigment of malarial parasites, CBC are performed.

Automated Hz detection by an instrument as used our study is a reliable diagnostic tool and correlates with disease severity. However, clinical usefulness as a prognostic tool is limited due to an overlap of pigment containing leukocyte (PCL) numbers recorded in severe versus non-severe malaria[2].

Haemozoin (Hz), the end product of the detoxification of haem, is phagocytosed by monocytes and granulocytes. Some studies have reported a link between Hz and dyserythopoiesis and anaemia[3,4]. Using microscopic enumeration of Hz-containing leukocytes (PCL), others have found a strong correlation between these cells and severity of malaria[5]. However, as has been pointed out before most of these studies suffer from two significant limitations that is the relative paucity of PCL and the rather low number of total leukocytes observed, thus causing a high statistical imprecision of microscopically determined

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counts and the microscopical counting of PCL is very time-consuming and subjective. Yet, another aspect of PCL is that their detection may be a very useful tool to diagnose malaria[6]. Flowcytometric analyzer is a rapid, high throughput device which needs less expertization for the diagnosis of malaria. Hence, it can be used in the diagnostic laboratories as an early modality for diagnosis of malaria in suspected as well as clinically in apparent cases.

This study was also designed to determine the effecacy of the automated hematology analyzer having Multi Angled Polarized Scatter Separation (MAPSS) based flow cytometric principals in the detection of malarial pigment hemozoin in malarial patient.

MATERIAL AND METHODS

This was a descriptive study carried out at North West General Hospital and Reasearch Centre (NWGH & RC). All requests received for complete blood count to NWGH & RC from July 2011 to September 2011 were included in the study. A total of 10032 consecutive complete blood counts were performed by automated hematology analyzer. Out of these 144 (1.43%) cases were flagged for malaria by Multi Angled Polarized Scatter Separation (MAPSS) based flow cytometry detection of hemozoin containing leukocytes. Thick and thin smears were prepared and stained with Giemsa .Comparison of Multi Angled Polarized Scatter Separation (MAPSS) based flow cytometry detection of hemozoin containing leukocytes was done with thin and thick film microscopy of giemsa stained smear**.** Thorough microscopic examination of the MAPSS positive detected cases for confirmation and species detection was done at Hayatabad Medical Complex and Khyber Teaching Hospital as well. Data was analyzed and results obtained in the forms of frequency tables and charts.

RESULTS

A total of 10032 consecutive complete blood counts were done. Total of 144 (1.43%) cases of malaria were diagnosed by gold standard microscopy. 132 cases were vivax species (91.66%) and remaining 12 cases (8.3%) were falciparum species.

On the CBC, the Multi Angled Polarized Scatter Separation (MAPSS) flagged 104 cases. (72.2%), of which 40 cases could be typed as, 38(95%) of plasmodium vivax and 2 cases (5%) plasmodium falciparum. Out of those 144 (1.43%) cases of malaria were diagnosed. The species specific frequencies are shown in Table 1.

Table 1: Total cases of Malaria diagnosed by microscopy

Species of malaria	No. of cases and percentage
Vivax species	132(91.7%)
Falciparum species	12(8.3%)
Total cases:	144(100%)

DISCUSSION

Parasitological diagnosis of malaria has for long time been based on microscopic detection of asexual malaria parasites on blood smear. Microscopy is still considered as the gold standard[7,8]. However many factors like the time of taking the blood sample, the quality of slide preparation and staining and labor intensiveness can affect the quality of microscopic diagnosis and requires considerable technical expertise. Intelligent and correct laboratory diagnosis and up-front identification of risk factors for progression to severe disease are the basis for optimal management of malaria. Recently, a number of alternative diagnostic approaches have evolved, including detection of Plasmodium species, DNA stained with acridine orange in a quantitative buffy coat analysis, PCR methods and assays based on detection of circulating Plasmodium species-specific antigens (e.g., P. falciparum histidine-rich protein 2) Many efforts have been made in the technological development for rapid and quantitative diagnosis of malaria[9,10,11,12].

Recent studies using automated hematology analyzers have demonstrated unexpected abnormalities in differential white blood cell plots and reticulocyte histograms from patients with malaria. In our study,s out of 144 (1.43%) cases of malaria diagnosed,132 cases were vivax species (91.66%) and remaining 12 cases (8.3%) were falciparum species. Sarta muhabtara et al also reported that out of 70 positive cases [49/70 (70%) P. vivax, 18/70 (25.7%) P. falciparum, and 3/70 (4.2%) both P. vivax and P.falciparum], 52 showed abnormal scattergrams by the analyzer. The sensitivity and specificity of hematology analyzer was found to be 74.2% and 88%, respectively[13]. Malarial anemia involves destruction of parasitized and non-parasitized red blood cells and dyserythropoiesis. Malarial pigment, hemozoin (HZ), is possibly implicated in[14].

It was further observed that on multi Angled Polarized Scatter Separation (MAPSS) for the the flagged 104 cases (72.2%), 40 cases could be typed as 38 (95%) of plasmodium vivax and 2cases (5%) plasmodium falciparum. The abnormal scattergrams were observed as double neutrophil, double eosinophil, grey zone, extended neutrophil zone with a decrease space between eosinophil and neutrophil, and a combination of above patterns.

CONCLUSION

Flowcytometric analyzer is a rapid, high throughput device which needs less expertise for the diagnosis of malaria. Hence, it can be used in the

diagnostic laboratories as an early modality for diagnosis of malaria in suspected as well as clinically in apparent cases. Multi Angled Polarized Scatter Separation (MAPSS) based flow cytometric principals for detection of malarial pigment hemozoin is of great importance in malaria endemic countries.

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