FREQUENCY OF WBC FLAGS GENERATED BY AUTOMATED HEMATOLOGY ANALYZER IN DIAGNOSED CASES OF DENGUE INFECTION

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ABSTRACT

Objective: To determine the frequency of WBC flags generated by automated hematology analyzers in cases of dengue diagnosed by serological tests. We also aim to compare the platelet count in the presence of different WBC flags.

Material and Methods: It was a cross sectional study, conducted at Chughtai Institute of Pathology. Total 1007 blood samples of serologically confirmed dengue patients in EDTA vial were obtained over a period of 03 months from September,2021 to November, 2021.These samples were run through Mindray BC-6800 which displayed flags for white blood cells. WBC flags displayed were analyzed in correlation with findings of CBC and peripheral smear through careful statistical analysis of the observed parameters.

Results: An abnormal WBC flag was displayed in all patients. The most common flag among these was atypical lymphocytes in 671 (66.6%) samples, followed by atypical lymphocytes + basophilia in 136 (13.5%), Atypical lymphocytes + neutropenia in 66 (6.6%), Atypical lymphocytes + lymphocytosis in 47 (4.7%), Atypical lymphocytes + lymphopenia in 31 (3.1%), Lymphopenia in 29 (2.9%), Atypical lymphocytes + monocytosis in 15 (1.5%), Lymphopenia + neutropenia in9 (0.9%) and Neutropenia in 3 (0.3%) samples.

Conclusion: WBC flags generated by automated hematology analyzer in a suspected dengue patient, especially during dengue epidemics can be used as a screening tool and can help in avoiding unnecessary serological testing.

Key Words: Dengue, NS1 antigen, WBC flags, Automated hematology analyzer.

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INTRODUCTION

Dengue is a viral infection that is caused by dengue virus (DENV) and transmitted to humans through bites of infected Aedes aegypti mosquitoes. Dengue is widespread throughout the world especially in tropical and subtropical climates. Dengue is endemic in Pakistan with many outbreaks during the last few years. The disease varies in severity from asymptomatic to mild cases. In few cases, severe flu like illness progressing to lethal complication called severe dengue can occur. Clinically, it is difficult to differentiate it from other diseases causing fever [1]. It is estimated that 390 million dengue virus infections occur per year, of which 96 million (67–136 million) are symptomatic (with any severity of disease) [2]. Another study estimated that 3.9 billion people are at risk of infection with dengue viruses. The actual burden of the disease is 70% in Asia [2]. The number of dengue cases reported to WHO increased 8-fold over the last two decades. The number of reported cases increased from 505,430 cases in 2000, to over 2.4 million in 2010, and 5.2 million in 2019. Reported deaths between the year 2000 and 2015 increased from 960 to 4032 [3].

The outbreak of dengue is common in Pakistan from the month of July to October. The National institute of health (NIH), Islamabad, reported 22938 dengue fever cases in Pakistan in 2017, more than 3,200 in 2018, almost 24,547 in 2019, and 3,442 cases in 2020. Pakistani health officials continue to report a surge in dengue fever cases, with roughly 25,478 cases reported through October 2021 [4]. Diagnosis of dengue based on the clinical presentation of the patient remains difficult for doctors in areas where diagnostic facilities are limited and it is difficult to differentiate it from other febrile illnesses like malaria and typhoid [5]. Early suspicion based on clinical presentation and baseline investigations is important for the timely diagnosis and effective treatment of dengue patients [6]. This requires the need of a screening tool that can predict the disease so that only necessary investigations can be requested accordingly. This can help in improving the disease outcome by early diagnosis and avoiding unnecessary investigations especially during endemics.

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Complete blood count (CBC) and peripheral blood film (PBF) is an important part of initial workup. The usual observed findings are thrombocytopenia and presence of atypical lymphocytes on smear [7]. The automated hematology analyzers have proved to be more effective in assessing hematological parameters thus replacing the manual methods. In addition to the blood cell counts and indices, these analyzers also provide information about the morphologic features of blood cells by generating abnormal "cell flags"[8]. These warning signs alert the operator to detect any abnormality and to respond accordingly.

There are several studies that have showed the correlation of WBC flags generated by hematology analyzers with blasts cells in PBF of leukemia patients [9,10]. However, very limited data is available in literature about the frequency and use of different WBC flags in infections like dengue and malaria. The aim of our study was to assess the reliability of WBC flags generated by automated hematology analyzers in cases of dengue diagnosed by serological tests.

MATERIAL AND METHODS

This was a descriptive cross-sectional study that was conducted in the Hematology Department of Chughtai Institute of Pathology, Lahore over a period of 3 months from September,2021 to November, 2021. Approval was obtained from the ethical and research committee of the institute (IRB letter No. CIP / IRB / 1087). Random sampling technique was used to include total 1007 samples of both male and female patients of all age groups who had nonstructural protein 1 (NS1) positive confirmed dengue infection. The sample size was calculated using OpenEpi, Version 3, open-source calculator using WBC flag as a reference parameter [11]. Peripheral blood sample of the patients was collected in ethylenediaminetetraacetic acid (EDTA) tube in volume of 2ml and was run on Mindray BC-6800 six-part hematology analyzer. CBC parameters including hemoglobin, hematocrit, WBC count, platelet count, absolute neutrophil count, absolute lymphocyte count, absolute monocyte count, absolute eosinophil count and absolute basophil count were noted. Any abnormal WBC flag was analyzed and correlated with peripheral blood film (PBF). Samples of suspected dengue patients who were NS1 negative and those who were NS1 positive but showed no WBC flags were excluded from the study. Data were entered into EXCEL worksheets and checked manually and corrected where necessary. Statistical analysis was done using social sciences (SPSS) version 20:00. Mean ± SD was calculated for continuous variable. Frequency and percentage was calculated for categorical variables. ANOVA and Post Hoc Test were applied on the collected data.

RESULTS

The age range among 1007 patients was 8–85 years with a mean of 40.67 years. Total number of male patients was n=817 (81.1%) and female patients was n=190 (18.9%). Table-I shows hematological parameters observed in all patients. Hematocrit was increased in 458 (45.4%) patients. Leukopenia (WBC count $<4 \times 10^9$/L) was detected in 387 (38.4%) patients. Basophilia was observed in 136 (13.5%) patients, followed by neutropenia in 66 (6.6%) patients. Thrombocytopenia (platelet count $<150 \times 10^9$/L) was observed in all patients (100%) at diagnosis with platelet counts $<50 \times 10^9$/L in 880 (87.3%) patients and $>50 \times 10^9$/L in 127 (12.6%) patients. WBC flags generated were noted. The frequencies of different WBC flags are summarized in Table-II. No blast cell was seen on peripheral blood examination in any case. Reactive lymphocytes were observed in 85% of the patient samples. Reactive lymphocytes are medium to large in size with more abundant amount of basophilic cytoplasm and irregular nucleus with coarse clumped chromatin. An abnormal WBC flag was seen in all 1007 patients. The most common flag among these was atypical lymphocytes in 671 (66.6%) samples, followed by atypical lymphocytes + basophilia in 136 (13.5%), atypical lymphocytes + neutropenia in 66 (6.6%), atypical lymphocytes + lymphocytosis in 47 (4.7%), Atypical lymphocytes + lymphopenia in 31 (3.1%), Lymphopenia in 29 (2.9%), atypical lymphocytes + monocytosis in 15 (1.5%), lymphopenia + neutropenia in 9 (0.9%) and Neutropenia in 3 (0.3%) samples. ANOVA and post Hoc test were applied to analyze the variance and relationship between independent (WBC flags) and dependent (platelet count) variable which came out to be significant i.e., <0.05 Table-III and Table-IV.

Table-I: Hematological parameters.

Parameters	Mean ± SD	Range
Hb (g/dl)	15.75±6.52	12-20
HCT (%)	46.92±5.34	35-62
TLC ($\times 10^9$/L)	5.83±3.17	1.00-16
Platelets ($\times 10^9$/L)	27.51±26.04	1.00-150
ANC	2.57±1.61	1.00-13
ALC	2.38±1.44	1.00-10
AMC	0.49±0.59	1.00-6.00
AEC	0.09±0.121	0.00-3.00
ABC	0.79±0.204	0.00-1.00

Hb-Hemoglobin, HCT-Hematocrit, TLC-Total leucocyte count, ANC-Absolute neutrophil count, ALC-Absolute lymphocyte count, AMC-Absolute monocyte count, AEC-Absolute eosinophil count, ABC-Absolute basophil count.

Table-II: Frequency and percentage of WBC flags displayed by automated hematology analyzer.

WBC Flags	Frequency	Percentage
Atypical lymphocyte	671	66.6%
Atypical lymphocyte, basophilia	136	13.5%
Atypical lymphocyte, neutropenia	66	6.6%
Atypical lymphocyte, lymphocytosis	47	4.7%
Atypical lymphocyte, lymphopenia	31	3.1%
Lymphopenia	29	2.9%
Atypical lymphocyte, Monocytosis	15	1.5%
Lymphopenia, neutropenia	9	0.9%
Neutropenia	3	0.3%
Total	**1007**	**100%**

Table-III: Relationship between platelet count and WBC flags.

WBC Flags	Platelet count (Mean)	Std. Deviation	Std. Error
Atypical lymphocyte	26.14	23.712	.915
Atypical lymphocyte, basophilia	18.80	15.948	1.368
Atypical lymphocyte, neutropenia	39.98	35.478	4.367
Atypical lymphocyte, lymphocytosis	25.55	18.602	2.713
Atypical lymphocyte, lymphopenia	43.97	36.043	6.474
Lymphopenia	51.24	45.319	8.416
Atypical lymphocyte, monocytosis	23.87	11.413	2.947
Lymphopenia, neutropenia	34.56	43.463	14.488
Neutropenia	82.00	44.911	25.929
Total	**27.51**	**26.048**	**0.821**

Table-IV: Comparison of mean platelet count in different WBC flags.

M	N	Mean Difference (M-N)	Significance
Atypical lymphocyte	Atypical lymphocyte, basophilia	7.339	.002
	Atypical lymphocyte, neutropenia	-13.845	.000
	Atypical lymphocyte, lymphocytosis	0.587	.877
	Atypical lymphocyte, lymphopenia	-17.828	.000
	Lymphopenia	-25.101	.000
	Atypical lymphocyte, monocytosis	2.273	.728
	Lymphopenia, neutropenia	-8.415	.317
	Neutropenia	-55.860	.000

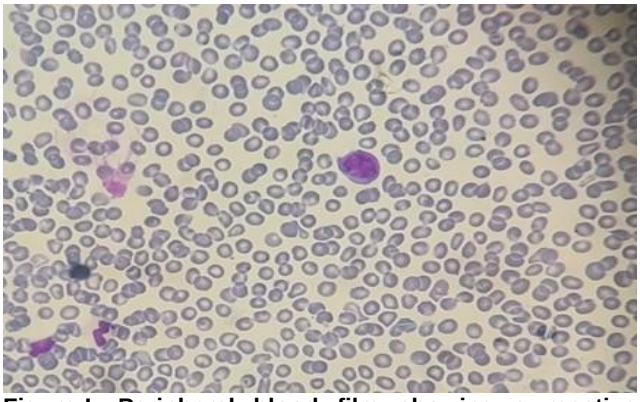

Figure-I: Peripheral blood film showing a reactive lymphocyte having an irregular nucleus and deeply basophilic cytoplasm (Giemsa stain, ×1000).

DISCUSSION

Dengue is one of the most common causes of fever in Pakistan [12]. Investigations of this disease include CBC, PBF, IgM/IgG antibodies against dengue virus and NS1 antigen detection. In resource limited countries like ours, white blood cell flags generated by automated hematology analyzers can be very helpful in diagnosing dengue infection and also they can be of prognostic value by predicting the severity of thrombocytopenia. Therefore, these WBC flags can help the practitioners to predict the severity of disease and thus taking preventive measures for the patient so that the disease does not progress to dengue hemorrhagic fever or dengue shock syndrome. Automated hematology analyzers are the automated instruments that provide valuable information about complete blood counts. The 3-part analyzer works on Coulter's Principle to differentiate between 3 types of WBC's, neutrophils, lymphocytes, and monocytes. In contrast, a 5-Part differential cell counter utilizes both Coulter's Principle and flow cytometry to differentiate between 5 types of WBC's, neutrophils, eosinophils, basophils, lymphocytes, and monocytes [13]. These analyzers not only give blood cell counts and indices but also generate out many flags. Though these flags are present in the print out but usually are ignored and not given any attention by the laboratory technologist during sample running as well as the Pathologist during reporting [14]. These flags may alert about the underlying disease keeping in mind the blood cell counts, clinical history of the patient and PBF findings.

There are several studies that have showed the correlation of WBC flags generated by hematology analyzers with blasts cells in PBF of leukemia patients [9,10]. Very little data is available about the frequency and the use of the WBC flags in different infections like dengue and malaria. In dengue and malaria endemic countries, these

infections pose a greater public health burden resulting in significant morbidity and mortality. In these countries, identifying WBC flags generated by automated hematology analyzers and correlating them with the blood counts, PBF findings and clinical picture of the patient can help in the early detection and timely management of these infections. Mishra *et al*. compared leucocyte cell population data of 319 cases of acute leukemia with 100 healthy and 52 reactive controls, using Sysmex XN1000 hematology analyzer [10]. Nishimura *et al*. observed different parameters generated by a three-part analyzer LC-667G CRP (HORIBA) for detecting malaria in patients presenting with fever [15]. Bhatti *et al* also noted similar abnormalities in WBC scattergrams of diagnosed cases of malaria [16].

Roy *et al*. [17] studied WBC flags in 28 diagnosed cases (16 females and 12 males) of dengue infection and found an abnormal WBC flag in all the patients. Observed findings were thrombocytopenia in 23 (82%) patients, leucopenia in 10 (35.7%) patients, relative lymphocytosis in 18 (64.3%) patients and increased hematocrit in 4 (14.3%) patients.

In contrast to this, our study included 1007 dengue patients with male to female ratio 4:1. Observed findings were thrombocytopenia in all patients (100%), leukopenia in 387 (38.4%) patients, basophilia in 136 (13.5%) patients, neutropenia in 66 (6.6%) patients and increased hematocrit in 458 (45.4%) patients. We also noted that 880 (87.3%) patients had severe thrombocytopenia with platelet counts <50 × 109/L at diagnosis while 127 (12.6%) patients had platelet counts >50 × 109/L. WBC flags generated were noted. The most frequent WBC flag was atypical lymphocyte (66.6%), followed by others. All these flags are indicative of dengue infection which were generated owing to the presence of reactive lymphocytes in dengue infection. No blast cell was seen on peripheral blood examination. Reactive lymphocytes were observed in 85% of the patient samples. Reactive lymphocytes are medium to large in size with more abundant amount of basophilic cytoplasm and irregular nucleus with coarse clumped chromatin. These reactive lymphocytes are relatively resistant to lysis and are spuriously counted as basophils, atypical lymphocytes and/or monocytes because of different morphological features of these lymphocytes including plasmacytoid lymphocytes and basophilic tailing of cytoplasm. These morphological features can be appreciated on peripheral smear [17,18]. There is a significant difference in mean platelet count noted with various WBC flags using ANOVA. we found that the lowest mean platelet count was associated with “atypical lymphocyte + basophilia” flag (18.8±15.9) followed by atypical lymphocyte + monocytosis (23.8±11.4) and atypical lymphocytes + lymphocytosis (25.5±18.6). Post hoc test was applied to find the significant difference in mean platelet count between specific groups. A P-value of <0.005 was considered significant. It was noted that there was a significant difference in mean platelet count between atypical lymphocyte flag and other flags like atypical lymphocytes + basophilia, atypical lymphocytes + neutropenia, atypical lymphocytes + lymphopenia, Lymphopenia and neutropenia. Thus, WBC flags generated by automated hematology analyzers can also be used as a screening tool in detecting the severity of thrombocytopenia in dengue infection.

CONCLUSION

Advanced technologies like automated hematology analyzers are designed to identify many hematological abnormalities, thus decreasing the need of verification of results on peripheral smears especially in centers with increased work load. However, in hospitals with limited resources where serological tests like Dengue IgG and IgM by ELISA and NS1 antigen are not available, an abnormal WBC flag generated by an automated hematology analyzer, either a basic three part or five parts, can be used as a screening tool for infections like dengue. This can help in avoiding unnecessary testing during epidemics and referring only suspected cases for confirmatory serological tests. Also, severity of the disease can be assessed thus allowing treating physicians to take timely decisions for the effective management of the patient.

AUTHOR CONTRIBUTION

Ayesha Younas: Literature search, data collection and article writing.

Mavra Fatima: Data collection and statistical analysis.

Ayisha Imran: Drafted the study design and proof reading.

Nauman Aslam Malik: Overall supervision of the study.

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