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Pseudobasophilia: A Helpful Screening Tool in Diagnosis of Dengue

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ABSTRACT

Introduction: Pakistan is dealing with an epidemic situation of dengue. Serological testing for its diagnosis is not available everywhere across the country. So, in the current scenario, basophilia flagged by automated hematology analyzer can be a helpful screening tool in early diagnosis and prognosis of dengue in a resource limited country like ours.

Aims & Objectives: To assess the utilization of basophilia flag as a screening tool in early diagnosis of dengue by studying the frequency of basophilia flag and it's prognostic significance by correlating absolute basophil count with severity of thrombocytopenia.

Place and duration of study: It was a cross sectional study and conducted at Chughtai Institute of Pathology from August 2021 to October 2021.

Material & Methods: Total 1007 patients who had NS1 positive confirmed dengue infection were included in the study and EDTA blood samples were run on Mindray BC-6800 six-part hematology analyzer. Basophilia flag was noted, its frequency was calculated and expressed as percentage. Also, correlation of absolute basophil count with platelet count was calculated. Statistical analysis was performed using SPSS 23.0, p value <0.05 was taken as significant.

Results: WBC flag showing "basophilia" was seen in 136 patients (13.6%) and a significant correlation was seen between raised absolute basophil count and thrombocytopenia using Pearson test.

Conclusion: Pseudobasophilia is an important screening tool in diagnosing dengue patients and as significant prognostic marker as increased absolute basophilic count correlates with severity of thrombocytopenia.

Key words: Dengue, NS1 antigen, Pseudobasophilia, Automated hematology analyzer.

INTRODUCTION

Dengue fever is becoming more prevalent in Pakistan as well as globally.[1] There has been a history of outbreaks of dengue fever in country since 1994 when first confirmed outbreak of dengue fever occurred. The next one happened in 2010 and recently we suffered from this epidemic in 2021. Dengue is a mosquito borne disease caused by dengue virus.[2] Patients commonly present with nausea, vomiting, high grade fever, joint pains (hence the name bone breaking fever) and skin rashes during febrile phase. This phase is followed by critical phase and recovery phase.[3]

Basic testing for dengue includes Complete blood count (CBC) which reveals changes in its parameters including Hemoglobin (Hb), Hematocrit (Hct), Platelet count, Mean platelet volume (MPV), Total white cell count (TLC), differential count including neutrophils, lymphocytes, monocytes, eosinophils and basophils (percentages as well as absolute counts). Differential white cell count can be helpful in diagnosis and prognosis of Dengue in resource limited areas.[4] Certain white cell flags generated by automated hematology analyzers can be a helpful screening tool in diagnosing and assessing the severity of dengue fever.[5]

A high percentage or absolute count of basophils is defined as Basophilia. It is commonly seen in allergic and acute inflammatory conditions and is also a characteristic feature of chronic myeloid leukemia.[6] The instruments detect basophils by flowcytometry using a semi conductor laser.[7] Pseudobasophilia is indicated by cells other than basophils which can be seen in bone

marrow infiltration and myeloma .[8] In Pakistan, the phenomenon of pseudobasophilia is appreciated owing to the presence of reactive lymphocytes.[9]

MATERIAL AND METHODS

A cross sectional study was conducted at Chughtai institute of Pathology from August 2021 to October 2021.Approval was obtained from the ethical and research committee of the institute. Total 1000 patients, both males and females of all age groups, who had NS1 positive confirmed dengue infection were included in the study. Two ml of peripheral blood sample was taken from each patient in EDTA tube following standard procedures. Samples were run on Mindray BC-6800 six part hematology analyzer. Basophilia flag was noted and correlated with peripheral smear examination. Suspected dengue patients who were NS1 negative were excluded from this study. Statistical analysis was done using SPSS 23.0. Frequencies were calculated and expressed as percentages. Correlation of absolute basophil count and severity of thrombocytopenia was observed using Pearson test. P value <0.05 was taken as significant.

Statistical analysis:
Data analysis was done using SPSS 23.0. Frequencies were calculated and expressed as percentages. Correlation of absolute basophil count and severity of thrombocytopenia was observed using Pearson test. P value <0.05 was taken as significant.

RESULTS

817 male, 190 female (total 1007) dengue patients were included in the study with mean age 40.77±15.58 years (Table-1). CBC showed mean Hb 15.75±6.5 g/dl, Hct 46.9±5.3 %, TLC 5.8±3.1 x 10^9/l, platelet count 27.5±26.04 x 10^9/l, Absolut Basophil Count (ABC) 0.08±0.23 (Table-2). A significant correlation (p = <0.004) was observed between increased Absolute Basophil Count (ABC) and fall in platelet count using Pearson test. (Table-3). Our study showed 136 dengue samples with flagging "basophilia" but when peripheral smears were prepared from these samples, there was no increase in basophils (hence the term pseudobasophilia). However, reactive lymphocytes with basophilic cytoplasm were seen on the smears which were falsely counted as basophils by automated hematology analyzer.

Characteristics	**Values**
Mean age (years)	40.77±15.58
Male	817 (81.1%)
Female	190 (18.9%)

Table-1: Mean age and gender frequency of patients

	Hb (g/dl)	**HCT (%)**	**TLC (×10^9/L)**	**Platelets (×10^9/L)**	**ABC**
Mean	15.75	46.92	5.83	27.51	0.0889
SD	6.527	5.347	3.178	26.048	0.235

Table-2: Hematological parameters
Hb: hemoglobin, HCT: hematocrit, TLC: total leukocyte count, ABC: absolute basophil count

		Platelets	ABC
Platelets	Pearson Correlation	1	-.119**
	Sig. (2-tailed)		.000
	N	1007	1007
ABC	Pearson Correlation	-.119**	1
	Sig. (2-tailed)	.000	
	N	1007	1007
**Correlation is significant at the 0.01 level (2-tailed).			

Table-3: Correlation between ABC (absolute basophil count) and thrombocytopenia

DISCUSSION

CBCs of 1007 dengue patients who were confirmed NS1 positive were analyzed which showed significant findings like rise in Hb, Hct, ABC and decrease in white cell count and platelet count. Along with these parameters, "basophilia" flags generated by automated hematology analyzer Mindray BC6800 were analyzed.

A higher hemoglobin and hematocrit level in dengue is due to increased vascular permeability; with the highest values seen on day 7.[10] A study by Martina et al, showed that the factors resulting in plasma leakage are cytokine storm and cross reactivity of anti-NS1 antibodies which result in the apoptosis of the endothelial cells.[11]

Basophilia is generally seen in conditions like infections, inflammation and also in myeloid neoplasms.[12] However in dengue patients basophilia flag suggests presence of atypical or

reactive lymphocytes owing to the infective process. Hence, the instrument gives falsely raised basophil count; that's why the term pseudobasophilia is used. To confirm this finding, peripheral smear should be prepared and examined carefully. In dengue patients, basophilia flag gives a hint of infection which can be further confirmed on peripheral smear where basophilic reactive lymphocytes can be appreciated.[13] Our study showed 136 dengue samples with flagging "basophilia" but when peripheral smears were prepared from these samples, there was no increase in basophils (hence the term pseudobasophilia). However, reactive lymphocytes with basophilic cytoplasm were seen on the smears which were falsely counted as basophils by automated hematology analyzer.

In a study from India, 52.9% dengue cases had basophils more than 2%.[14] In a research carried out in Thailand, basophil count was within normal range.[10] In another endemic area, 91.2% dengue cases flagged pseudobasophilia and thrombocytopenia when the samples were run on Sysmex XE-2100 analyzer. However, when peripheral smears of such cases were examined; the only significant finding was reactive lymphocytes.[15] The variability of the basophil count depends on the time of sample collection. Researches reveal a weak synchroneity between instruments regarding the basophil count.[8,16]

Pseudobasophilia in dengue is an underreported phenomenon which was most commonly associated with flags of "atypical lymphocytes" and "blasts" given by the hematology instruments.[17] Our study also shows similar results with high frequency of basophilia along with atypical lymphocytes flag on Mindray BC6800. Increased ABC also showed correlation with severity of thrombocytopenia i.e with increasing absolute basophil count there was a marked decrease in platelet count.

CONCLUSION

Pseudobasophilia can be used as a helpful tool in the diagnosis of dengue in a resource restricted country like Pakistan where serological confirmation of the disease is costly and not easily available across the country. Also early detection of severe thrombocytopenia by using basophil flag in dengue patients can prevent them from bleeding complications.

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