Original Article

Differential Leukocyte and Platelet Counts as Early Diagnostic Indicators in Acute Dengue Infection

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[1,2,3] *Conception of study*
[1,2] *Experimentation/Study conduction*
[1,2,3] *Analysis/Interpretation/Discussion*
[1] *Manuscript Writing*
[2,3] *Critical Review*
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Abstract

Introduction: Dengue is an epidemic in Pakistan. Proper monitoring of dengue cases is necessary for its early diagnosis and prevention, especially in developing countries with limited resources. Our study aims to use some hematological parameters as Early Diagnostic Indicators in Acute Dengue Infection which can help in reducing mortality and morbidity.

Materials & Methods: Dengue NS1 Antigen test was used to identify 119 Dengue Patients over the months of August-October, 2019. Hematological data from such patients were collected and analyzed.

Results: In our study thrombocytopenia was prominent with sensitivity based on gender distribution being 86% for males and 76% for females. According to age, distribution thrombocytopenia had a sensitivity of up to 81% in the younger age group and 80% in the older age group. The other prominent findings in younger age groups were lymphopenia, neutropenia, and monocytopenia with a sensitivity of 55%, 43%, and 24% respectively. Lymphopenia and neutropenia had similar sensitivity across the two genders, whereas monocytopenia had higher sensitivity among females of 27% as opposed to males of 12%. Eosinopenia did not differ as much among the two age groups i.e. 18% in young and 19% in older patients and across the two genders i.e. 56% in males and 60% in females. The sensitivity of lymphocytosis in older patients is 21% which was also significant.

Conclusions: The Differential Leukocyte Count and Platelets Count can help as early indicators of acute dengue infection in resource-limited areas. This can help in early diagnosis and prompt treatment thus reducing complications.

Keywords: Acute dengue, differential leukocyte count, platelets count, thrombocytopenia, neutropenia

Introduction

Dengue is a mosquito-borne viral infection transmitted by the mosquito Aedes Aegypti. Dengue Virus (DENV) is responsible for causing this viral infection. There is a possibility of getting infected four times as there are four DENV serotypes. Mild illnesses are produced by DENV infections. Acute flu-like illness is usually caused by DENV. This could develop into a severe deadly complication in certain cases also known as severe dengue. Not many treatment options are available for dengue/severe dengue. Early detection and prompt treatment can reduce fatality rates of severe dengue below 1%.[1]

Almost 100 million people are affected by Dengue fever worldwide.[2] Dengue fever is an epidemic in Asia including India, Srilanka, Maldives, Pakistan, and China. The tropical climate supports the spread of the dengue virus by mosquitoes.[3]

In Pakistan, Dengue is a major health issue and is an epidemic that has been occurring every year since 2006.[4] Punjab, Sindh, and Khyber Pakhtunkhwa are the provinces in Pakistan where dengue cases are mostly seen.[5] The increase in population and urbanization are contributing factors that have led to the dengue epidemic.[6] In the 2011 outbreak of dengue fever in Pakistan, Lahore was the most affected city, suffering from almost 290 deaths. The study conducted in Pakistan showed that all four dengue serotypes were responsible for the outbreak of dengue in Punjab.[8] Dengue fever is a self-limiting disease from which many patients recover. However, in some cases, it leads to severity resulting in dengue hemorrhagic fever which could lead to high mortality.[7] Proper monitoring of dengue cases is necessary for its prevention. For early diagnosis, there should be an effective diagnostic strategy.[9]

Differential blood counts and their evaluation can help the diagnosis of dengue fever, especially in an endemic resource-limited area.[10] The hematological profile is necessary to evaluate for early diagnosis of dengue fever. Early diagnosis aids in prompt treatment so that patient is treated before the disease progresses and results in more complications.[11]

The rapidly increasing number and severity of Dengue cases in the last half-century demand the need for early diagnosis and prevention of Dengue Infection. Due to a lack of proper sanitation, hygiene, and prophylaxis, Dengue Infection has been notably seen more in underdeveloped countries. Early intervention may be life-saving. Clinical symptoms of dengue infection include nausea, vomiting, rash, aches, a positive tourniquet test, and leukopenia. In severe cases following symptoms mucosal bleeding, lethargy, restlessness, and liver enlargement can be seen.

The combination of these clinical symptoms defines dengue in a person living in a dengue-endemic area.[12] Different diagnostic tests are available for dengue infection which includes virus isolation in cell culture, detection of viral RNA by nucleic acid amplification tests (NAAT), or detection of viral antigens by ELISA or rapid tests. These can be diagnosed during the febrile period.[13] Most patients in underdeveloped countries lack the resources to get these tests done. However, a complete blood count is easy to do and is accessible to all.

Our study aims to use hematological parameters for early diagnosis of acute dengue infection which can help in reducing mortality and morbidity associated with this infection. As hematological parameters can be assessed through a simple complete blood complete count test which is cost-effective, rapid, and readily available in a community hospital. Secondly, this study will also assist in diagnosing early dengue infection

Materials and Methods

This is a retrospective cross-sectional study done in the Hematology/Pathology Department of Fauji Foundation Hospital/Foundation University Islamabad during July-November, 2019. Retrospective records of patients with acute dengue infection were retrieved from Medix (Health information software) used at our hospital by adding the search words Dengue, CBC, and NS1. 119 patients were found as having acute dengue infection from July- November 2019 at our hospital.

For CBC two milliliters of venous blood were collected in EDTA and were analyzed using Sysmex XE- 2100 (Sysmex Corporation, Kobe, Japan) automated hematology cell analyzer for complete blood counts including WBC, platelets, and differential leucocyte counts. Where required a peripheral film was made stained with Leishman stain to confirm DLC. Reference ranges for the hematological parameters that were used in our study are as follows. The normal reference range of blood counts is as follows:

- Neutrophils - 2-7 X 10 9 /L
- Lymphocytes – 1-3 X 10 9 /L
- Monocytes - 0.2-1 X 10 9 /L
- Eosinophils - 0.02-0.5 X 10 9/L
- Normal Platelet Count: 150-400 × 109 /L

For diagnosing Dengue, the NS1 Antigen test was used to identify dengue-positive patients. The test was done by using Humasis Dengue NS1 antigen Testing Kit. Nonstructural protein NS1 is secreted into the blood during dengue infection. Dengue NS1 Antigen test is an immunochromatographic assay.19 It uses a monoclonal antibody specific to Dengue NS1 Antigen for accurate determination of Dengue virus infection. The test used serum, plasma, or whole blood. Whole blood was stored at 2-8 °C and the specimen was used within 3 days. If it was stored for longer, the specimen was frozen and stored below approximately 20°C. The test procedure involved pulling out the specimen and device and leaving it at room temperature for 15 minutes before the test. Furthermore, 3 drops (100uL) of whole blood, plasma, or serum were taken by pipette or dropper and dropped in the specimen insertion hole. Results are shown after approximately 15-20 minutes. Dengue virus antigen, in serum/plasma or whole blood samples, is allowed to react with the anti-Dengue NS1 monoclonal antibody-coupled gold conjugate followed by reaction with anti-Dengue NS1 monoclonal antibody in the test lines. The presence of an evident line on the membrane in the test region indicates a positive Dengue NS1 antigen test. Whereas, a negative test result is indicated by the absence of coloured bands.

Data was recorded on Medix software by hospital staff. The type of data was quantitative. The data was collected, reformed, and arranged. All participants irrespective of gender, race, or ethnicity tested positive for the NS1 Detection Test at FFH during the time period of data collection. Patients aged 14-70 were included in the study. Patients below 14 years of age or above 70 years of age were excluded from the study. Individuals who were NS1 negative or who were not tested for the NS1 Detection Test were also excluded from the study. Patients with bone marrow failure, chemotherapy, and drugs were excluded from the study.

The data was analyzed by using Excel Version 2013 for windows. Descriptive statistics were performed. Categorical data are presented as frequency & percentage. Quantitative variables are presented as mean ± SD.

Ethical consideration: The study was approved by the Ethics Committee of FFH and permission to access data was taken.

Results

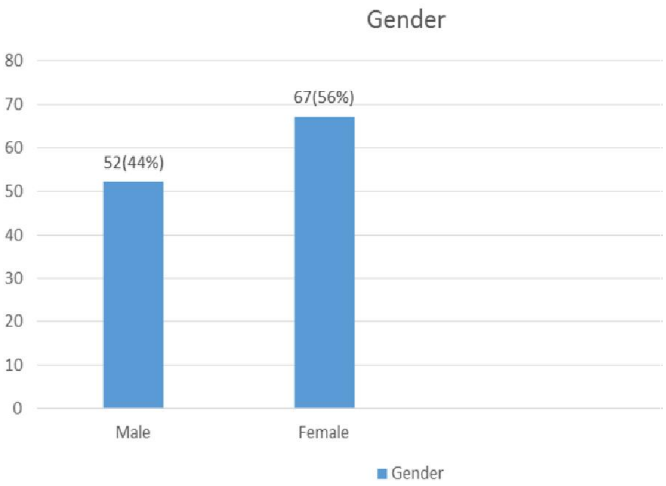

Figure 1: Gender distribution of patients

Table 1: Sensitivity of hematological parameters based on gender

Blood Parameters	*Gender*	*Sensitivity (In Percentage)*
Thrombocytopenia	Females	76
	Males	86
Neutropenia	Females	42
	Males	37
Lymphopenia	Females	58
	Males	52
Eosinopenia	Females	60
	Males	56
Monocytopenia	Females	27
	Males	12
Thrombocytosis	Females	0
	Males	0
Neutrophilia	Females	4
	Males	2
Lymphocytosis	Females	1
	Males	2
Eosinophilia	Females	0
	Males	0
Monocytosis	Females	1
	Males	6

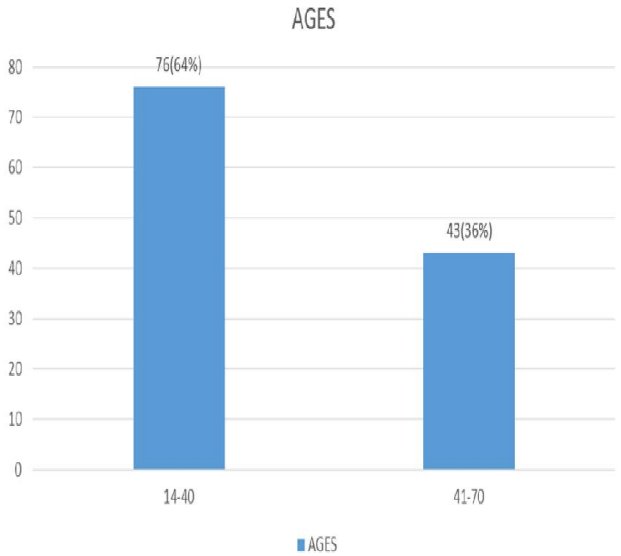

Figure 2: Sex distribution of patients

Table 2: Sensitivity of hematological parameters according to Age Groups

Blood Parameter	*Age Range*	*Sensitivity (In Percentage)*
Thrombocytopenia	14-40	80
	41-70	81
Neutropenia	14-40	43
	41-70	32
Lymphopenia	14-40	55
	41-70	53
Eosinopenia	14-40	18
	41-70	19
Monocytopenia	14-40	24
	41-70	14
Thrombophilia	14-40	0
	41-70	0
Neutrophilia	14-40	4
	41-70	2
Lymphocytosis	14-40	4
	41-70	21
Eosinophilia	14-40	1
	41-70	0
Monocytosis	14-40	5
	41-70	0

Discussion

Our study showed an age range of 14 to 70 years with a median age of 34 years. There was a slight female predominance with a male to female ratio of 1:1.29. This gender ratio was unlike other studies where there was male predominance.[14,15] This may be because Fauji Foundation Hospital caters to Veteran army wives and children.

Thrombocytopenia was the most prominent finding. The sensitivity of thrombocytopenia based on gender distribution was found to be 76% for females and 86% for males.

According to age distribution thrombocytopenia had a sensitivity of up to 81% in the younger age group (Group I) and 80% in the older age group (Group II).

The other prominent findings were neutropenia and lymphopenia. Both neutropenia and lymphopenia were found in younger age groups with a sensitivity of 43% and 55% respectively. Both of the findings had similar sensitivity across the two genders.

Monocytopenia was also found in younger age groups compared to older age groups 24% Vs 14%. Monocytopenia was also a relevant finding appearing more in females with a sensitivity of 27% as opposed to males having a sensitivity of 12%.

Eosinopenia, however, did not differ between the two age groups. Young patients had a sensitivity of 18% whereas old patients had a sensitivity of 19%. Eosinopenia appeared to be another important finding with a sensitivity of 60% in females and 56% in males.

Lymphocytosis was also a significant finding among older patients with a sensitivity of 21%. The sensitivity of neutrophilia, monocytosis, eosinophilia, and Thrombocytosis was not significant.

The most significantly altered hematological parameter in acute dengue infection was platelets. In males, the sensitivity was 86% and in females, it was 76%. Thrombocytopenia was more common in males than in females. It is in accordance with many studies.[7,14,15,16] The bone marrow suppression and the antibody-mediated response of destruction of platelets can lead to thrombocytopenia.[15] More attention should be given to patients with low platelet count in the early acute phase of dengue as it is an indicator that the disease can progress to a more severe case like dengue hemorrhagic fever, if not treated timely.[7]

Neutropenia was the second most significant hematological parameter that was deranged in acute dengue infection. An interesting finding was that neutropenia was present in the younger age group (17-40 years) as compared to the older age group (40-70 years). This is in accordance with other research where neutropenia has been reported in the literature.[10,17,18] Females between the ages of 14 to 40 years had higher sensitivity to neutropenia.

We had 30% of cases with neutropenia in concordance with few other studies.[18] However, few studies showed a higher proportion of cases.[19] We didn't

observe any cases of severe neutropenia but few studies had a higher proportion of severe neutropenia.[17]

Neutropenia is attributed to a decrease in granulocytes due to bone marrow suppression in the early phase and degeneration of mature neutrophils with the shift to the left in the febrile phase.[12]

Sensitivity of Eosinopenia was more in females than males, which was 60%. Eosinophilia was not present in acute dengue infection. This lower proportion of eosinophilia is in accordance with some studies. 10 Eosinopenia was seen in the acute phase of the disease 20 and a rebound increase in eosinophils was seen with an increase in absolute eosinophil count.[16,21]

In our study, Lymphopenia was found in 55% of cases between 14 to 40 years and 53% in ages between 41 to 70 years. Lymphopenia needs to be further explored in early dengue fever. Lymphocytosis was comparatively less in our study than seen in other studies.[10,16] Lymphocytosis was seen in older patients which was about 21%. Lymphopenia was more common in females. In some studies, lymphocytes were reported to be within a normal range.[7] Lymphopenia might be seen due to the infectious nature of dengue Infection, malnutrition, or because of the presence of other infectious diseases such as Tuberculosis, HIV, etc. which is quite prevalent in Pakistan.[18]

Lymphocytosis with Plasmacytoid lymphocytes is a response to viral antigens followed by transformation to plasmacytoid lymphocytes to control the spread of infection and represents enhanced immunoglobulin production in Dengue.[22] Plasmacytoid lymphocytes provide a diagnostic clue and aid in the differential diagnosis of Dengue.[22,23]

It was observed that atypical lymphocytes could serve as a marker of disease activity and are present in a higher number of cases in DHF rather than DF.[23]

Severe Dengue is marked by T and B cell activation and apoptosis. Activation of 'T' cells results in the release of inflammatory cytokines triggering the death of cells through apoptosis which contributes to severe dengue.[24,25]

The sensitivity of monocytosis was less as compared to monocytopenia. The sensitivity of monocytosis was found to be only 6% which is in accordance with some studies.[10]

In some other studies, there were high cases of monocytosis.[16] Monocytopenia was up to 27% in this study. We believe that the factors inducing lymphopenia and monocytopenia could be similar.

Literature on differential Leukocyte count in early dengue infection is still scarce. Our study has thrown light on differential leukocyte counts as an early indicator of acute dengue infection. Our study has relevance for community-based and under-resourced settings in developing and underdeveloped countries. In Pakistan, which is a developing country with few resources, it is necessary to find easy and simple diagnostic methods to evaluate dengue patients. Dengue infection, if left untreated can lead to serious complications with high mortality and morbidity. So, the differential blood counts can assist in the early detection of this infection

Limitations: As it was a retrospective study, the gender distribution is not uniform as our hospital caters to primarily female patients. Another limitation is the limited sample size.

Conclusion

Early diagnosis and prompt treatment are necessary for patients with acute dengue infection. The presence of thrombocytopenia, neutropenia and eosinopenia will help as early indicators of acute dengue infection. Lymphopenia and monocytopenia can also assist in the early detection of acute dengue infection.

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