HIGH FLUORESCENCE LYMPHOCYTE COUNT AS AN EARLY PREDICTOR OF SEVERE THROMBOCYTOPENIA IN DENGUE INFECTION

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ABSTRACT

Objective: Dengue infection (DI) is the most common arthropod-borne viral disease with symptoms ranging from mild flu like illness to fatal hemorrhagic fever. Thrombocytopenia is one of the common manifestations of dengue which can lead to life threatening bleeding. Currently, there is no validated test available that can predict the severity of thrombocytopenia in a dengue patient. Dengue has been well associated with presence of high fluorescence lymphocytes in the peripheral blood. High fluorescence lymphocyte count (HFLC) is a parameter generated at no extra cost when an automated complete blood count (CBC) is performed on Sysmex XN-1000 automated hematology analyzer. We aim to assess the significance and correlation of the HFLC with thrombocytopenia during the course of dengue infection especially during day 1 to 5 of the infection.

Material and Methods: This is a cross sectional study and was conducted at Chughtai Institute of Pathology from August 2021 to October 2021. Total 312 patients, both males and females, who had NS1 positive confirmed dengue infection were included in the study. Their platelet count and HFLC were noted at the time of diagnosis using Sysmex XN-1000. Follow up CBC were analyzed for next 5 days to observe the severity of thrombocytopenia.

Results: There is significant negative correlation between absolute HFLC and platelet count on day one to five of the infection. On receiver operator characteristic curve analysis, we found that HFLC < 0.5 x103/μL had 60% sensitivity and 71.8% specificity for severe thrombocytopenia (platelet count less than 50 x 103/μL on day 5 of the infection.

Conclusion: HFLC is a negative predictive factor for severe thrombocytopenia and therefore can be used as an early predictor of disease progression.

Key Words: Dengue infection, NS1 antigen, High fluorescence lymphocyte count, Thrombocytopenia, Automated hematology analyzer.

INTRODUCTION

Dengue infection is an arthropod borne viral infection which is caused by an infected female Aedes aegypti mosquito. There are four serotypes of dengue virus (DEN 1, DEN 2, DEN 3, DEN 4) which can cause symptomatically similar disease [1]. The symptoms can vary from mild to severe depending upon the type and number of times a patient has been infected by a specific serotype. Usually when a patient is infected by a single serotype, he acquires immunity against that specific serotype but when he is again infected by another serotype the symptoms aggravate and can lead to severe disease and its complications [2]. The common symptoms of the disease include fever, nausea, vomiting, body aches and rash whereas severe symptoms include severe abdominal pain, persistent vomiting, rapid breathing, bleeding gums and hematemesis.

Dengue infection affects around 2.5 billion people around the globe and the combined impact of dengue and covid -19 can have devastating impact on the health system of a country [3]. Currently with no available vaccine for dengue infection, vast variability in symptoms of the disease and added burden on the health system due to prevailing covid 19 pandemic it is important to have a cost effective and readily available laboratory parameter to predict the disease severity in a dengue patient.

In today's world of automation, hematology analyzers have proven to be an excellent tool to calculate the leukocyte differential counts of the peripheral blood. These automated analyzers have reduced the need for manual counts which not only saves time but also provide accurate results specially when numerous samples are received by the laboratory at the time of health crisis [4]. Sysmex XN 1000 is one of such automated hematology analyzers which gives differential count of leukocytes which include eosinophils, basophils, neutrophils, lymphocytes and monocytes. Apart from these Sysmex XN 1000 has an additional feature that

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provides the differential count of immature granulocytes, nucleated red blood cells and high fluorescence lymphocyte count (HFLC). HFLC is a hematological parameter that is calculated on basis of high nucleic acid in RNA of a reactive lymphocyte [5]. In various researches it has been found out that HFLC is high in infectious diseases such as dengue infection.

Most viral infections initiate the immune response that leads to the activation of lymphocytes. These activated lymphocytes are known as reactive lymphocytes and often the term atypical lymphocyte is used synonymously for them. These atypical lymphocytes are larger in size with abundant basophilic cytoplasm and clumped nuclear chromatin. HFLC and atypical lymphocytes have been shown to have significant correlation between them thus HFLC is parameter that can be further explored in viral infections such as dengue [6].

Dengue is characterized by leucopenia followed by thrombocytopenia [7]. The severity of thrombocytopenia is one of the major factors that determine the outcome of the disease. The severity of thrombocytopenia can be graded depending upon the platelet count of the patient. It is broadly classified into three categories namely mild, moderate and severe. In mild thrombocytopenia platelet count is between 100,000 to 150,000, moderate thrombocytopenia is when platelet count is between 50,000 to 100,000 and severe thrombocytopenia is when platelet count drops below 50,000. Severe thrombocytopenia can lead to life threatening bleeding thereby it should be monitored very closely [8].

Till date there is no validated hematological parameter available that can precisely predict the severity of thrombocytopenia in a dengue patient. Therefore, there must be a- cost effective, readily available and easy to perform test that can used as the predictive factor for thrombocytopenia in the course of dengue infection. Thus, the aim of the study is to see whether HFLC can be used to predict the severity of thrombocytopenia in a dengue patient thereby helping clinicians in timely management of the disease.

MATERIAL AND METHODS

This is a cross sectional study that was conducted at Chughtai Institute of Pathology, Lahore after getting approval from the ethical and research committee of the institute. The duration of the study was from August, 2021 to October, 2021. A total of 312 patients, both male and female of all age groups, who had tested positive for non-structural protein (NS 1 antigen) were included in the study using non probability consecutive sampling. The sample size was calculated using OpenEpi, Version 3, open-source calculator taking high fluorescence lymphocyte count as reference parameter [9]. 2 ml of venous blood sample was taken from each patient in Ethylenediamine tetra acetic acid (EDTA) tube following standard sampling procedures. After which the samples were run on Sysmex XN-1000 automated hematology analyzer and complete blood counts including platelet count, total leucocyte count, hemoglobin, hematocrit and HFLC were extracted. No additional reagent was used to calculate HFLC. Thereby, the platelet counts of the selected patients were analyzed for the next 5 days using Sysmex XN-1000 to see the severity of thrombocytopenia. All the patients who had tested negative for NS1 antigen and who had previous history of any hematological malignancy, viral hepatitis or HIV were excluded from the study. Data were entered in the excel work sheets, checked manually and logically corrected where needed. The data was analyzed using SPSS 20.0 and mean values were calculated. Spearmen test was used to calculate the p value.

RESULTS

We included 312 NS 1 antigen positive patients in our study. Out of these 312 patients, 212 (67.7%) were males whereas 101 (32.3%) were females. The average age of our study group was 45.09 ± 18.4 years. The mean hemoglobin level at the time of presentation was 14.73 ± 2.1 g/dL, mean hematocrit was 44.59 ± 5.6 % and mean TLC was 5.6 ± 3.2 x 10³/µL. Of the total 312 patients included in the study, 58.6% (183) had HFLC < 0.5 x 10³/µL whereas 41.4% (129) had HFLC ≥ 0.5 x 10³/µL. The average absolute HFLC of our study group was 0.53 ± 0.8 x 10³/µL.

In our study we found out that there is statistically significant negative correlation between absolute HFLC and platelet count at day 1 to day 5 of the disease. The p value was calculated using Spearmen's correlation which is less than 0.05 (Table-I). The area under the curve for HFLC is 0.718 (Figure-I). ROC analysis was done to determine the predictive value of HFLC < 0.5 x10³/µL for severe thrombocytopenia on day 5 of the infection. The results showed that HFLC had 60% sensitivity and 71.8% specificity for severe thrombocytopenia (platelet count less than 50 x 10³/µL) on day 5 of the infection.

Table-I: HFLC as a significant predictive factor for severe thrombocytopenia.

Days of Infections	Spearmen Correlation coefficient	P value
1	-0.12	0.02
2	-0.14	0.01
3	-0.20	<0.001
4	-0.27	<0.001
5	-0.37	<0.001
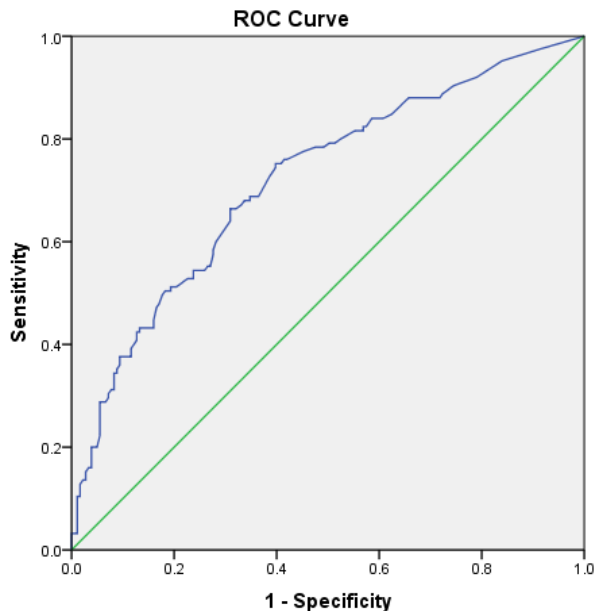
Diagonal segments are produced by ties.

Figure-I: Receiver operator characteristic curve (ROC) curve analysis showing HFLC < 0.5 x10³/µL as a predictive cutoff for severe thrombocytopenia (platelet count less than 50 x 10³/µL).

DISCUSSION

Dengue is prevalent in Pakistan since 2005 with its worse outbreak in 2011. The geographical location, climate changes, ever growing population, increase in unplanned urbanization and lack of efficient vector control programs have all contributed in making dengue endemic in Pakistan [10].

A study conducted in Uttar Pradesh; India stated that males are more prone to be affected by dengue as compared to the females. In their study the male to female ratio of dengue positive cases was 1.5:1 [11]. This finding is consistent with our finding as well. In our study we observed that males are more prone to dengue as compared to the females probably due to their outdoor work routine which expose them to the mosquitos [12].

A study conducted at Chiang Mai University Hospital analyzed the blood parameters in a dengue patient. According to this study the mean hemoglobin and hematocrit values at day 1 of infection was 13.41g/dL (12.99-13.94) and 40.94% (39.61-42.32) whereas the mean hemoglobin levels in our study group was 14.73±2.1 g/dL and hematocrit was

44.59±5.6 % [13]. The reason for raised hemoglobin and hematocrit is increased capillary permeability and leakage through the capillary endothelium [14].

In the study carried out in Ziauddin Hospital, Karachi the mean leucocyte counts at day 1 of dengue infection was found out to be 3.7±1.62 x 10^3/µL whereas in our study the mean of total leucocyte counts at day 1 is 5.6±3.2 x 10^3/µL[14]. Generally, the patients with dengue infection present with leucopenia but there is no significant correlation between the degree of leucopenia and thrombocytopenia [16].

In the study conducted by Raharjo and Hadi in Indonesia, they found out that the HFLC count was increased in patients with dengue hemorrhagic fever [5]. They also found out the normal value of HFLC which was between 0.0-1.4%. In our study also the correlation of HFLC is significant when compared to the platelet counts of day 1 to 5 of the infection thereby suggesting that the HFLC counts are high in dengue positive patients.

A cross sectional study conducted at National Institute of Blood disease and Bone marrow transplantation, Karachi compared the HFLC among dengue patients and those suspected to have immune thrombocytopenia (ITP). They found out that patients with dengue had HFLC 11.71 ± 7.17% while those with ITP had HFLC 0.198 ± 0.25%. This signifies that dengue patients with thrombocytopenia have markedly increased HFLC as compared to patients with immune mediated thrombocytopenia [17].

As per our knowledge there is no research conducted in our country that has compared the HFLC with the severity of thrombocytopenia in dengue patients. This study is conducted to check whether HFLC can be used to as a predictive factor for severity of thrombocytopenia in dengue patients. This parameter can be added in the CBC reports of dengue patients so that attending physicians can predict the severity of the disease and its complications to take preventive measures for the patient.

CONCLUSION

We concluded that HFLC is a negative predictive factor for severe thrombocytopenia and can be used as an early predictor of disease progression. The high levels of HFLC at the time of diagnosis of dengue infection will indicate that there are high chances of developing severe thrombocytopenia related complications later in the course of the disease.

This parameter is not widely available in our country so further researches regarding this parameter should be conducted to utilize its ability to predict severity of thrombocytopenia in dengue infection. Once this parameter is validated, it can be a very helpful tool in predicting hemorrhagic complications in dengue patients and will help clinicians in timely management of the disease.

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AUTHOR CONTRIBUTION

Isma Imtiaz: Data collection and literature search.
Aiman Mahmood Minhas: Article writing and data collection.
Mavra Fatima: Statistical analysis and proof reading
Ayisha Imran: Drafted the study design.
Nauman Aslam Malik: Overall supervision of the study.
Akhtar Sohail Chughtai: Overall supervision of the study.

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