Original Research Article

High fluorescence lymphocyte count in dengue

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Abstract

Background: High fluorescence lymphocyte count (HFLC) is a research parameter indicating the presence of high fluorescence lymphocytes, which represent activated cells (antibody-secreting B- lymphocytes/plasma cells). HFLC in dengue fever is used to estimate the cellular immune response through the presence of blue plasma lymphocytes. **Aims:** To determine the HFLC in patients with dengue fever using hematology analyser, and to assess the severity of the disease based of the percentage of HFLC. **Methods and Material:** The present study was conducted in the department of Pathology in a tertiary care hospital. Eighty dengue positive cases and 20 controls were included in the study**.** HFLC values were obtained from the automated SYSMEX XN 1000 analyser. Statistical analysis of data was done by SPSS23 software. **Results:** Kruskal Wallis test showed significant difference in platelets between dengue fever, dengue hemorrhagic cases and control groups($p<0.01$). Average HFLC% count in dengue fever group is 1.7 and in dengue hemorrhagic fever it is 18.85 and there exist significant difference between them with $p<0.01$. **Conclusions:** Increase in HFLC values can be related to the pathogenesis of dengue hemorrhagic fever. The immunological response can be detected by an increase in High Fluorescent Lymphocytes Count (HFLC) which can be observed in the automated Sysmex XN-1000 hematology analyzer. Thus HFLC can be used to assess the severity of the disease, at an early stage.

Keywords: SYSMEX-XN 1000, Dengue, High fluorescence lymphocyte count

Introduction

Dengue virus (DENV) is the most widespread arbovirus in the world, with endemic dengue transmission found in over 128 countries and about 4 billion people living in areas at risk of infection. Dengue can be identified and confirmed clinically using anti-DENV antibodies, non-structural protein 1 (NS1) antigen, or DENV-specific nucleic acid detection, among other approaches[1].

Dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) are reversible vascular complications of Dengue. They cause extreme thrombocytopenia, bleeding, and increased vascular permeability[2]. The Sysmex XN-1000 automated haematology analyzer (Sysmex America, Inc.) is a 5-part differential analyzer that is fully automatic (eosinophils, basophils, neutrophils, lymphocytes, and monocytes). Automation not only makes the diagnostic work setting more effective, but it also reduces inspection times. This allows for the establishment of diagnoses and treatments earlier[3]. Furthermore, the automatic blood cell counters are based on two fundamental principles: impedance and optical scatter. Coulter's impedance method, developed in 1950, is the most widely used method today.

The optical light scatter concept is used in the Sysmex XN-1000 haematology analyzer. The aim of using optical and hydrodynamic focusing detection with a laser beam is to measure and observe the size of cells. The presence of high fluorescence lymphocytes, which reflect activated cells (antibody-secreting B- lymphocytes/plasma cells), is indicated by the high fluorescence lymphocyte count (HFLC)[4].

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Aims and objectives

To determine the HFLC in patients with dengue fever using the hematology analyser Sysmex XN 1000

To assess the severity of the disease, based on the percentage of HFLC

Subjects and Method

In a tertiary care teaching hospital in Mangalore, India, a descriptive retrospective study was undertaken at the Department of Pathology for a period of one year, from July 2020 to July 2021. A total of 100 cases were gathered, with 80 confirmed Dengue positives and 20 healthy people serving as controls. The patients' information including age, gender and clinical characteristics was gathered and tallied.All the blood samples were assessed by the Sysmex XN-1000 automated hematological analyser. Various parameters like platelet count, hemoglobin value, total leucocyte count, haematocrit and High fluorescence lymphocyte count (HFLC) as a %age, were tabulated in Excel sheet.

Statistical analysis

Statistical analysis of data was done by IBM ® SPSS ® Statistics 23 (IBM Corporation, USA) software. Descriptive statistics, frequency, percentage, median and range were calculated and summarized. Based on normality test, parametric or non-parametric test were used to compare the **3 groups** – dengue fever, Dengue hemorrhagic fever and controls (ANOVA or Kruskal Wallis test). The level of significance was set at 5%.

Results

Out of the sample size of 100 cases, 20 cases comprised of healthy volunteers (controls) and 80 were confirmed dengue cases. Out of the 80 dengue positive cases, 20 patients showed signs and symptoms of Dengue haemorrhagic fever. These patients had variable degree of ascites, pleural effusion, breathlessness, fever, myalgia, headache, rash, hypotension and tachycardia. The other 60 patients showed mild symptoms, commonly being fever, myalgia and headache. 3 paediatric cases below the age of 12 years who showed signs and symptoms of Dengue shock syndrome died.Age distribution showed

that 20% participants were of age 0 to 20y, 41% of age 21 to 40, 29% of age 41 to 60 and the remaining 10% of age 61 to 80. 32% were female and 68% male.

Table 1:showing comparison of Platelets between the groups

GROUPS	DENGUE FEVER	DENGUE HEMORRHAGIC FEVER	CONTROL	RESULTS
MEDIAN	58,000	22,500	2,8,500	P<0.01
RANGE	6000-2,86,000	2000-64000	2,40,000-5,14,000	

Table 1: Kruskal Wallis test showed significant difference in platelets between dengue fever, dengue hemorrhagic cases and control groups ($p<0.01$). The comparison between uncomplicated dengue fever and the dengue hemorrhagic group showed average platelet count in dengue fever group of 58,000 and in dengue hemorrhagic fever it was 22,500. This is a significant difference between them, with $p < 0.01$. There was also significant difference between haemorrhagic cases (22,500) and controls (2,80,500) with $p < 0.01$. Comparison between dengue hemorrhagic cases and dengue fever group also showed significant difference in platelet count with $p < 0.01$. (Figure 1)

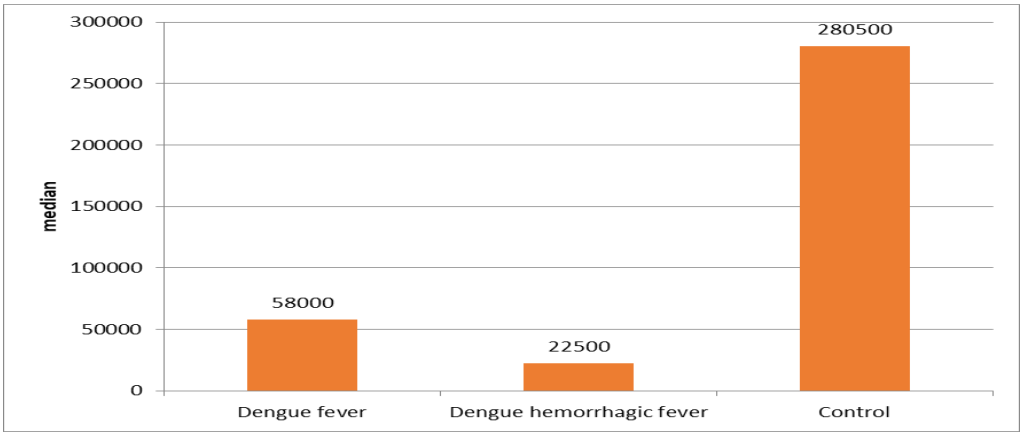

Fig 1: Comparison of platelet groups

On comparison of hematocrit (HCT) between the 3 groups, there was no significant difference ($p>0.05$).However there was slight increase in hemoconcentration among the dengue hemorrhagic cases compared to other 2 groups.

Table 2 showing comparison of HFLC% between the groups

GROUPS	DENGUE FEVER	DENGUE HEMORRHAGIC FEVER	CONTROL	RESULTS
MEDIAN	1.7	18.85	0.1	P<0.01
RANGE	0-18.7	10.2-29.2	0-0.5	

Table 2: Kruskal Wallis test showed a significant difference in HFLC% between dengue fever, dengue hemorrhagic fever and control groups ($p < 0.01$). In the comparison between dengue fever and dengue hemorrhagic fever, average HFLC% count in dengue fever group was 1.7 and in dengue hemorrhagic fever 18.85. There was thus a significant difference between dengue hemorrhagic fever (median=18.85) and control (median=0.1) with $p < 0.01$.

Comparison between 3 groups also shows significant difference in HFLC% count with $p<0.01$. (Figure 2)

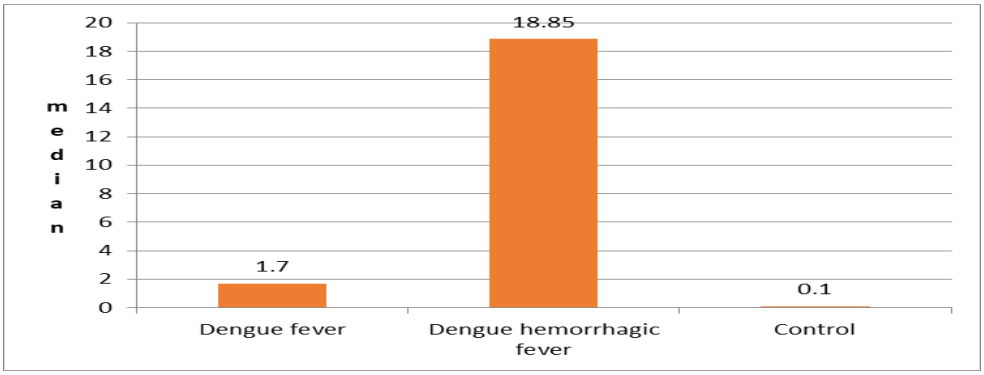

Fig 2: Comparison of HFLC between 3 groups

Discussion

Significant increase in HFLC % was seen among patients with Dengue hemorrhagic fever when compared to control cases in our study. Thrombocytopenia was significant among patients with DHF followed by dengue fever.

A study done by Raharjo et al. showed that HFLC values obtained from those 47 samples were from 2.0 to 32.3% with an average value of 11.6% and a standard deviation of 7.45%[3].

Linssen et al. discovered that B-lymphocytes, T-lymphocytes, big granular lymphocytes, activated monocytes were quantified in HFLC when an infection occurs, and antibodies are produced produced[4].

Dengue-related thrombocytopenia has been suggested to be immune-mediated response. It's likely that a higher Atypical lymphocyte count reflects more immunological dysregulation, leading to more severe thrombocytopenia. The more serious complications of DD, such as haemorrhage and shock, may share the same pathophysiology[5]. According to the aetiology of DHF, an increase in HFLC in DHF patients could mean that B-lymphocytes, T-lymphocytes, or monocytes are activated. Hence, an increase in HFLC values can be related to the pathogenesis of dengue hemorrhagic fever[3,6].A study done by Abeysuriya V et al. showed that Atypical lymphocyte count of $<0.5 \times 10^3$/L on admission may be a novel negative predictive factor for severe thrombocytopenia on day 5 to day 7 of Dengue disease[5].The presence of blue plasma lymphocytes is utilised to measure the cellular immune response in dengue fever using HFLC. The activation of B-lymphocytes, T-lymphocytes, or monocytes triggers the immune response to the dengue virus[7]. A rise in High Fluorescent Lymphocytes Count (HFLC) can be seen with the automated Sysmex XN-1000 haematological analyzer, indicating an immune response. Thus, the HFLC can be used to estimate the severity of dengue hemorrhagic conditions in this situation.

Conclusion

HFLC is a research parameter which can be used to evaluate the severity of conditions associated with Dengue fever. It indicates the immune response to the Dengue virus. Along with other parameters like platelet count, hematocrit value and serum ferritin, we can use HFLC values to quantify the risk and prognosis associated with the condition in a earlier stage. There has been little preceding published literature on this condition, and this study adds to the information on early diagnosis and management of Dengue fever-related conditions like the Dengue hemorrhagic fever and Dengue shock syndrome. It might thus help in preventing the morbidity and mortality associated with the disease.

Conflict of Interest: Nil Source of support: Nil

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