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CONTENTS

RESEARCH

LITERATURE REVIEW

CASE REPORT

HIGH FLUORESCENT LYMPHOCYTE COUNT EXAMINATION IN DENGUE HEMORRHAGIC PATIENTS WITH SYSMEX XN-1000 HEMATOLOGY ANALYZER

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ABSTRACT

Sysmex XN-1000 hematology analyzer is an automated 5-part diff analyzer (eosinophils, basophils, neutrophils, lymphocytes, and monocytes). In the calculated area, the type of difference between the Sysmex hematology device and other hematology devices is Immature Granulocyte (IG), Nucleated Red Blood Cell (NRBC), and High Fluorescent Lymphocytes Count (HFLC). The cells calculated in the HFLC area are atypical lymphocytes. In patients with dengue hemorrhagic fever, it is often found atypical lymphocytes called blue plasma lymphocytes. The purpose of this study was to determine the description of HFLC in patients with dengue fever using the hematology analyzer Sysmex XN-1000. A descriptive retrospective study was conducted during April-May 2017. The subjects of the study were adult patients diagnosed with dengue hemorrhagic fever with WHO criteria. Of the 47 samples of Dengue Hemorrhagic Fever (DHF) patients, the average HFLC results were between 2.0-32.3%, which was 11.5%, while the average range of normal HFLC values was between 0.0-1.4% and was 0.3%. In cases of DHF, there is an increase in HFLC. This is likely to be attributed to atypical lymphocyte increase in dengue hemorrhagic fever. Further research with more varied samples still needs to be done.

Key words: High fluorescent lymphocytes count, dengue hemorrhagic fever, atypical lymphocytes

INTRODUCTION

Sysmex XN-1000 hematology analyzer is an automated 5-part diff analyzer (eosinophils, basophils, neutrophils, lymphocytes, and monocytes). Since 1980, automated blood cell counters have begun to replace manual examinations because they have better precision and accuracy, except to calculate platelet counts. The automatic blood cell counters from various factories also have generally provided eight complete hematological parameters with a leukocyte type count that can be three parts, five parts, or six parts in less than a minute requiring less than 200 μL of blood. The automation not only makes workload setting more efficient but also shortens inspection times so that it is expected to help the establishment of a diagnosis and therapy earlier.[1-4]

The automated blood cell counters, moreover, has two basic principles namely impedance and optical scatter.[1-3] The impedance method made by Coulter in 1950 is the most commonly used method at present. Meanwhile, optical light scatter uses laser or non-laser light on automated blood cell counters.

The Sysmex XN-1000 hematology analyzer has an optical light scatter principle. The use of optical and hydrodynamic focusing detection aims to calculate and observe the size of cells using a laser beam. When the cell is through the sensing zone, the distribution of light is detected by the detector photos and converted into electrical signals. The number of signals produced is proportional to the number of cells that go through the sensing zone during a specific period.[2]

The old 3-part diff analyzer is useful enough for screening leukocyte counts. However, a 5-part diff analyzer or a 6-part diff one has better sensitivity and specificity to detect cell distribution and morphological abnormalities, such as blasts and nucleated erythrocytes.[1-5]

The Sysmex XN-1000 hematology analyzer is known to no longer be considered as a 5-part diff analyzer but become a 6-part diff analyzer. It means that six leukocyte types can be calculated by the Sysmex XN-1000 hematology analyzer, namely eosinophils, basophils, neutrophils, lymphocytes, monocytes, and immature granulocyte. With the calculation of IG, which includes promyelocytes, myelocytes, and metamyelocytes, the Sysmex XN-1000 hematology analyzer can be said to be a

hematology analyzer with a 6-part diff analyzer. This result distinguishes the Sysmex hematology analyzer from other hematology analyzer devices.[6]

The part diff analyzer area of Sysmex hematology analyzer has three hematological parameters, namely IG, Nucleated Red Blood Cell (NRBC), and HFLC. High fluorescent lymphocytes count calculation is based on the high fluorescence activity of atypical lymphocytes obtained from the part diff analyzer area. High fluorescent lymphocytes count value then can be determined based on the high nucleic acid content of RNA derived from lymphocytes. An increase in the number of HFLC indicates the occurrence of an infection or an immune response to an illness.[6]

On the other hand, dengue fever is still a problem of infectious diseases in the world, especially in the tropical countries. Based on data from the World Health Organization (WHO) there is an increase in the incidence of dengue infection each year. In 2004, the outbreak of dengue in Indonesia resulted in 58,301 cases with a Case Fatality Rate (CFR) of 1.1%. Dengue virus actually can stimulate an extensive clinical state. Based on the clinical condition resulted, WHO divides diseases caused by dengue virus into mild undifferentiated febrile illness, Dengue Fever (DF), Dengue Hemorrhagic Fever (DHF), and Dengue Shock Syndrome (DSS).[7]

Dengue virus has four serotype variants, namely DEN-1, DEN-2, DEN-3, and DEN-4. Dengue fever is an acute infectious disease caused by the dengue virus and characterized by symptoms of biphasic fever, myalgia, headache, pain in some parts of the body, rash, lymphadenopathy, and leukopenia. In most cases, DF is self-limited, but there is still a risk of progressively developing phase into DHF or DSS. Dengue virus infection can also trigger changes in the humoral immune system and cellular immune system.

The changes in the humoral immune system can be detected through the formation of IgG and IgM antibodies that can be identified through serological examination. Meanwhile, the changes in cellular immune system, according to some previous researchers, can be assessed by obtaining atypical lymphocytes that are typical for dengue infection, namely blue plasma lymphocytes considered as useful markers for early diagnosis with high sensitivity and specificity. It is because dengue virus infection can activate the immune system. Thus, impaired immune responses, such as inversion of the CD4/CD8 ratio not only can disrupt the ability of the immune system to clear the virus but also can cause excessive cytokine production which will affect T lymphocytes to differentiate into atypical lymphocytes, especially blue plasma lymphocytes.[8]

For those reasons, this research aimed to reveal whether HFLC would increase in DHF, since HFLC parameter in dengue fever could be used to estimate cellular immunity activities through the presence of blue plasma lymphocytes. This research used Sysmex XN-1000 hematology analyzer to evaluate HFLC values in patients.

METHODS

This research was a descriptive retrospective study conducted from April to May 2017. The subjects of this research were adult patients aged older 17 years old and diagnosed with dengue hemorrhagic fever according to WHO criteria. Serology test was also carried out to support the diagnosis of dengue hemorrhagic fever infection. Anamnesis, physical examination, and venous blood examination with HFLC then were performed on the subjects when they arrived at Mitra Keluarga Waru Hospital, Sidoarjo.

Moreover, clinical criteria for DHF used in this research were based on the WHO, such as an increase in hematocrit as much as >20% (hemoconcentration) or plasma leakage. Diagnosis of DHF then could be established if there were two clinical criteria plus one criterion for laboratory fulfilled (minimum increase in hematocrit), such as sudden fever for 2-7 days usually considered to be biphasic, bleeding tendency (petechiae, ecchymosis or purpura), thrombocytopenia ($<100,000/mm^3$), and/or leak plasma. Meanwhile, the diagnosis of DSS could be established if clinical DHF criteria were obtained plus circulation failure. Serologically, dengue infection could be detected if anti-dengue IgM and or IgG results were positive. Next, a regular blood test was performed to screen hemoglobin levels, hematocrit, platelet counts, and peripheral blood smear in DF suspect patients to detect the presence of relative lymphocytosis and blue plasma lymphocytes. Hence, HFLC examination was carried out using Sysmex XN-1000 on those patients diagnosed with dengue hemorrhagic fever based on the results of anamnesis, physical examination, and laboratory examination to detect their HFLC values. Before that, the normal range of HFLC values was determined by analyzing the blood cells of normal patients, and then its average value and standard deviation were measured.

Ethical clearance was obtained from the Medical Research Ethics Commission, Faculty of Medicine, Wijaya Kusuma University, Surabaya with number No.09/SLE/FK/UWKS/2019.

RESULTS AND DISCUSSION

During the period of research from April to May 2017, a range of normal HFLC values was determined from 48 healthy people who did medical check-ups. A range of normal HFLC values obtained was between 0.0 - 1.4% with an average value of 0.3% and a standard deviation of 2.71%.

In this research, 47 patients with dengue hemorrhagic fever became samples of the study. The frequency distribution of those patients by age then indicated that those patients were mostly 18 years old (19.6%). Meanwhile, the frequency distribution of patients by sex revealed that the patients were primarily female (52.2%) (Figure 1).

Furthermore, 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%.

Based on Figure 2, there was an increase in HFLC values of 47 samples compared to normal ones. The normal values of HFLC in the research were 0.0-1.4%, whereas in those patients with dengue hemorrhagic fever the values of HFLC were between 2.0-32.3% with an average value of 11.6% and a standard deviation of 7.45%. The increased HFLC values can be related to the pathogenesis of dengue hemorrhagic fever.

Previous research argued that there were two types of the opposite immune responses, immune responses to prevent and cure dengue virus infections and immunopathological responses triggering clinical manifestations of DHF. Dengue virus stimulates monocyte cells to produce IFN-γ from lymphocytes through non-specific immune responses. IFN-γ induction requires contact between monocytes infected with the dengue virus and these lymphocytes. Non-infected monocytes will not stimulate autologous lymphocytes to produce IFN-γ. IFN-γ will protect monocytes that have not been infected from the dengue virus.[9]

Subsequently, the dengue virus will stimulate B-lymphocytes to produce interferon (IFN) through a specific mononuclear immune response to antigens DEN-1, DEN-2, DEN-3, and DEN-4. B-lymphocytes give a high response to the DEN-3 antigen, but a low one to the DEN-1, DEN-2, and DEN-4 antigens. This process proves the occurrence of cross responses between each different serotype. As a result, formation of dengue virus and antibody complexes, activation of T lymphocyte, activation of the complement system, and production of pro-inflammatory and anti-inflammatory cytokines occur.[9]
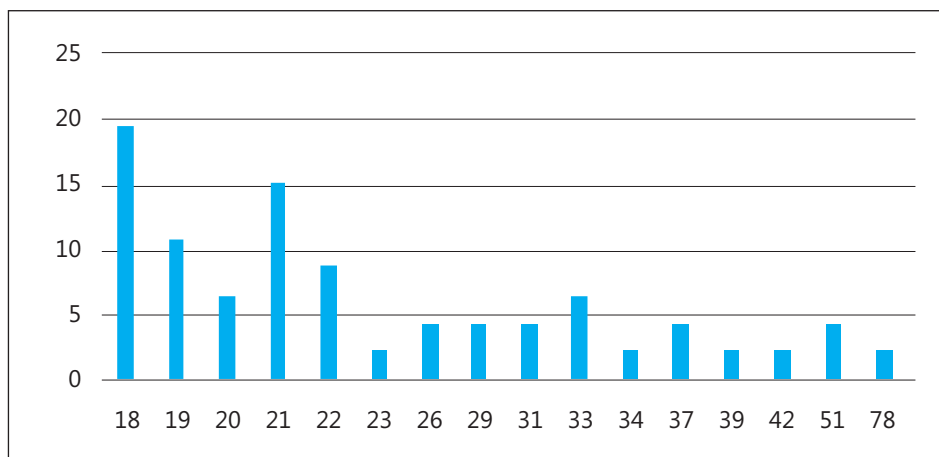
Figure 1. Diagram of patients with dengue hemorrhagic fever based on age
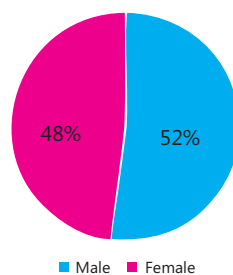
Figure 2. Frequency distribution of dengue hemorrhagic fever patients by sex

The creation of the dengue virus and antibody complexes occur when viruses circulating in the blood circulation, most of which will bind to IgG specific antibodies to form immune complexes. Some researchers even get 48-72% of DHF patients with immune complexes. The immune complexes are found on platelet surfaces, B-lymphocytes, capillary walls, and kidney glomeruli. The immune complexes attached to platelets by the liver and spleen reticuloendothelial cells can lead to thrombocytopenia. Examination of provoked platelet Aggregation Adenosine Diphosphate (ADP) then proves that there is impaired platelet function. Hence, the activation of immune complexes in the endothelium will result in activation of coagulation resulting in coagulation defects, and coupled with thrombocytopenia causing bleeding in DHF.[9]

T-lymphocytes play an essential role in the pathogenesis of DHF. Activation of T-lymphocytes will cause the release of mediators that play an essential role in capillary permeability and blood clotting cascade. In 20-50% of cases, lymphocyte transformation occurs, which then will affect lymphocyte activation. In the acute phase, T-lymphocytes will decrease and then will return to the normal one in the healing period. Immune status before illness, however, can also affect the activation of T-lymphocytes.[9]

Similarly, research conducted by Linssen *et al.* found that cells counted in HFLC were B-lymphocytes, T-lymphocytes, large granular lymphocytes, activated monocytes when an infection occurs and antibodies secreted.[10]

Therefore, an increase in HFLC in DHF patients can indicate that B-lymphocytes, T-lymphocytes, or monocytes are activated well according to the pathogenesis of DHF. But, during the healing period, activated lymphocytes will decrease and cause HFLC to decrease in number, along with an increase in the number of thrombocytes.

CONCLUSION AND SUGGESTIONS

Based on the results of this research, it can be concluded that in dengue hemorrhagic fever, an immunological response, both humoral immune response and cellular immune response, can occur. This immunological response is triggered by activation of B-lymphocytes, T-lymphocytes, or monocytes. 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. Nevertheless, this research was a descriptive study analyzing only HFLC results. Hence, further researches are suggested to relate HFLC images with days of thrombocyte decline in patients with dengue hemorrhagic fever.

REFERENCES

1. Longanbach S, Miers MK. Automated blood cell analysis. In: Keohane EM, Smith LJ, Walenga JM. Rodak hematology: Clinical principle and application. 5th Ed., Missouri, Elsevier, 2016; 208-28.
2. Instrumentation in hematology. In: Turgeon ML. Clinical hematology theory and procedures. 5th Ed., Florida, Lippincott Williams and Wilkins, 2011; 546-54.
3. Zsandencki M ato Sanduski?, Genevieve F, Gerard J, Gordon A. Spurious counts and spurious result on hematology analyzer: White blood cells, red blood cells, hemoglobin, red cell indices, and reticulocytes. In: Marchant KK, Davis BH. Laboratory hematology practice. The United States, Willey Black, 2012; 79-82.
4. Instrumentation in hematology. In: Turgeon ML. Clinical hematology: Theory and practices. 6th Ed., Florida, Lippincott Williams and Wilkins, 2017; 619-36.
5. Meintker L, Ringwald J, Rauh M, Krause SW. Comparison of automated differential blood cell counts from Abbott Saphire, Siemens Advia 120, Beckman Coulter DxH 800, and Sysmex XE-2100 in normal and pathologic samples. Am J Clin Pathol, 2013; 139(5): 641-50.
6. Sysmex. Counting of highly fluorescent lymphocytes (HFLC) as antibody-producing plasma cells on XE-5000. Sysmex extra online. 2011; 1-8.
7. Chandra A. Demam berdarah dengue: Epidemiologi, patogenesis, dan faktor risiko penularan. Aspirator, 2010; 2(2): 110-119.
8. Irianti DM, Reniarti L, MS A. Hubungan jumlah limfosit plasma biru dengan spektrum klinis dan perannya dalam memprediksi perubahan spektrum klinis infeksi dengue pada anak. Sari Pediatri, 2009; 10(5): 325-30.
9. Tjahjono G, Nasronudin. Imunopatogenesis demam berdarah dengue. In: Nasronudin. Penyakit infeksi di Indonesia. 2nd Ed., Surabaya, Airlangga University Press, 2011; 117-122.
10. Linssen J, Jennissen V, Hildmann J, Reisinger E, Schindler J, *et al*. Identification and quantification of high-fluorescence-stained lymphocytes as antibody synthesizing/secreting cells using the automated routine hematology analyzer XE2100. Clinical Cytometry Society, 2007; 72B: 157–166.