https://doi.org/10.48047/AFJBS.6.12.2024.5893-5898

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

A typical Lymphocyte Count as a significant prognostic indicator to assess the severity of Dengue infection: A hospital based study

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Article History
Volume 6, Issue 12, 2024
Received: 30 June 2024
Accepted: 20 July 2024
Doi:
10.48047/AFJBS.6.12.2024.5893-5898

Abstract

Background: Dengue infection is one of the most common viral infections in the developing countries. The early identification of patients at risk of severe dengue infection is very important to guide the management of infected patients. Atypical lymphocyte count (ALC) is a research parameter used in recent days which is cost effective and used along with an automated complete blood count (CBC). The purpose of this study is to assess the various platelet parameters in the patients infected with dengue virus and to assess the association of ALC with the severity of the infection. *Methods:* The study was conducted in blood samples of 96 patients who were diagnosed to have Dengue infection by ELISA method. Platelet parameters including platelet count and Mean Platelet Volume (MPV) were analysed. Peripheral smear was done in all cases and percentage of atypical lymphocyte count was calculated. Platelet count and atypical lymphocyte count were compared in all cases. *Results:* In the present study, maximum number of patients was between 10 and 20 years of age. There was variable thrombocytopenia observed among the cases, 12% of which showing severe thrombocytopenia with platelet count below 20,000 cells/cu.mm. MPV was found to be >8 fl in 73% of patients, which is a significant finding. It was also found that atypical lymphocyte count was more than 17% in 90% of Dengue cases. It was also observed in this study that as the platelet count decreased, Atypical Lymphocyte Count was higher ($p< 0.001$). Hence it was significant. *Conclusion:* It is observed in the present study that there is a significant inverse correlation between atypical lymphocyte count and the platelet count in Dengue patients. Hence it can be used as a simple and cost

effective tool to alert the clinicians and guide them in further management.
Keywords: Dengue, Atypical Lymphocyte Count, thrombocytopenia

Introduction

Dengue fever has become a major public health problem in our country. It is the leading arbovirus infection transmitted by Aedes mosquito and is on the rise worldwide, affecting nearly 2.5 billion people worldwide. The infection is caused by one of the four dengue virus serotypes- DEN-1, DEN-2, DEN-3 and DEN-4. These arboviruses belong to the family Flaviviridae [1]. The infection is a self-limiting disease and is characterized by fever, headache, rash, joint pain, nausea and vomiting [2]. Laboratory diagnostic methods for confirming dengue virus infection involve detection of viral nucleic acid, antigens or antibodies or a combination of these techniques. The tests commonly used are NS1, IgM antibody and IgG antibody using enzyme-linked immunosorbent assay, complete blood count (CBC) and peripheral blood smear (PBS) [3,4]. Platelet count, Mean platelet volume (MPV), platelet distribution width (PDW) and plateletcrit (PCT) are some of simple platelet indices used in diagnosis [4].

Platelet volume is an indicator of function of platelet and its activation. Normal range of mean platelet volume is 7.2 fl to 11.7 fl. The mean platelet volume (MPV) measures platelet activity. Thrombocytopenia is seen almost universally with dengue infection. This is the result of decreased production and increased destruction of platelets. Severe thrombocytopenia is thought to correlate with disease severity and may contribute to the risk of bleeding. The mean platelet volume (MPV) is affected by platelet aging and varies according to the balance between production and destruction [1].

During the progression of Dengue infection, affected patients are observed to produce atypical lymphocytes, which are observed in the peripheral circulation. These atypical lymphocytes have an increased nucleic acid content and scattering properties that can be detected by increasing the fluorescence signal. They are identified as CD19-B lymphocytes using flow cytometry [5].

Peripheral blood film (PBF) is a laboratory test that involves cytology of peripheral blood cells applied to a glass slide. PBF is invaluable in characterizing a wide range of clinical conditions. Despite advances in haematology automation and the application of molecular techniques, PBF remains a very important diagnostic test for haematologists. Atypical lymphocytes are morphologically distinct from adult lymphocytes in their heterogeneous size and shape. Few studies have concluded that the correlation between platelet count and atypical lymphocyte count plays an important role to assess the severity of Dengue infection.

In the present study, we have assessed the platelet parameters and emphasized the importance of atypical lymphocyte count as a prognostic indicator in the diagnosis of Dengue infection [6].

Materials and Methods

This is a cross-sectional study, done over a period of six months. 96 blood samples of all patients who were diagnosed to be Dengue positive were included in the study.

Inclusion criteria:

1. Dengue positive samples of patients in all ages and both sexes

Exclusion criteria:
1. Patients with thrombocytopenia of other causes.

Samples were taken in an EDTA vacutainer and complete blood hemogram was done using Sysmex XP-100 CBC analyser. The values of platelet indices such as platelet count and mean platelet value (MPV) were considered. Peripheral smears were also made using Leishman stain and the morphology of cells was analysed to look for Atypical Lymphocyte count. We correlated the atypical lymphocyte count with that of the platelet count using ANNOVA Test.

Results

In this study, the maximum number of patients was seen in the age group of 10 to 20 years and males (56%) were higher than females (44%). It was observed in this study that 12% of the patients had severe thrombocytopenia with platelet count less than 12,000 cells/cu.mm (Table 1), (Figure 1).

Table.1 Platelet count of Dengue cases in the present study

PLATELET COUNT (Cells/cu.mm)	PERCENTAGE (%)
< 20,000	12
20,000 – 50,000	15
50,000 – 1,00,000	16
1,00,000 – 1,50,000	27
1,50,000 – 2,00,000	30
>2,00,000	10

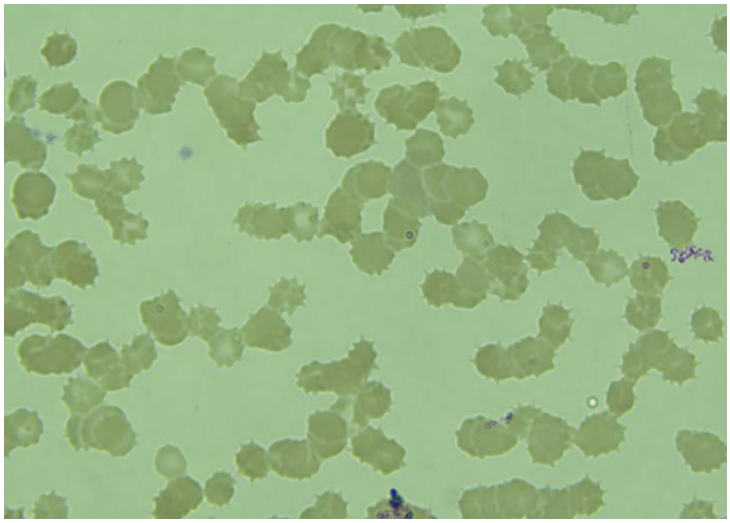

Figure 1. Peripheral smear of a patient with thrombocytopenia. (Leishman 10X)

Mean Platelet Volume (MPV) and total lymphocyte count were also analysed. It was found that MPV was significantly low in 73% of cases (Table 2) and total lymphocyte count was higher than normal in more than 50% of cases. (Table 3).

Table.2 MPV of Dengue cases in the present study

MPV (fl)	**Percentage (%)**
<8	27
>8	73

Table.3 Total lymphocyte count of Dengue cases in the present study

TOTAL LYMPHOCYTE COUNT (%)	Percentage(%)
<20	22
20-30	30
31-40	26
40-50	12
>50	10

This study also showed that the atypical lymphocyte count was more than 17% in 90% of Dengue cases, which is significantly high. Atypical lymphocytes were seen as large cells with abundant cytoplasm and irregular nucleus, mostly dispersed in the periphery of the smears (Figure 2)

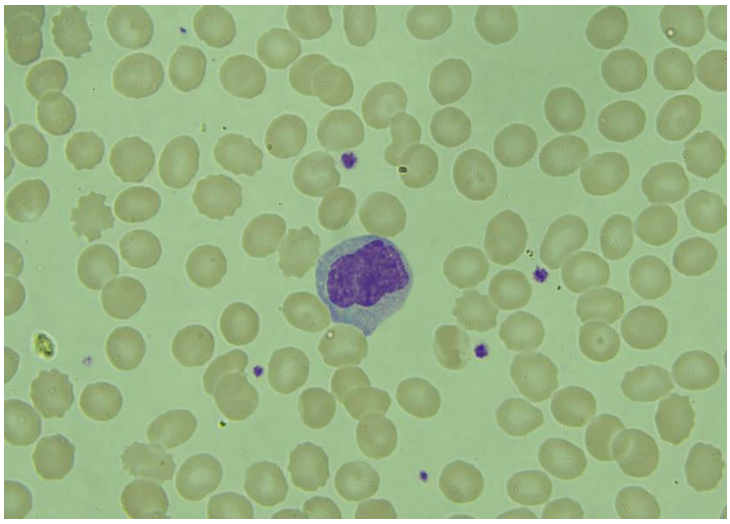

Figure 2. Peripheral smear of a Dengue patient showing atypical lymphocyte. (Leishman 40X)

It was also observed that there is a significant inverse correlation between the platelet count and atypical lymphocyte count (P = <0.001)

Discussion:

Dengue fever is a growing public health problem in the tropical countries across the world, with India being the front runner. It is estimated that there are currently 50-100 million cases of persistent dengue fever worldwide, which includes > 500,000 reported cases of dengue hemorrhagic fever and shock syndrome[7].

In a study conducted by Shetty et al., it was found that dengue fever was the most common cause of febrile thrombocytopenia studied in the hematology laboratory. Serological diagnosis of dengue viral infection is routinely carried out by detecting NS-1 antigen and dengue viral antibodies in the patient's serum depending on the day of illness using a commercial kit[8].

According to a study conducted by Kulkarni RD, Patil SS et al., dengue specific IgG/IgM detection has long been the mainstay of diagnosis. Dengue specific antibodies only appear in primary infection around the fifth day of fever. Even with most secondary infections, IgM and IgG antibodies can only be detected on the third day. Therefore, in primary and secondary dengue infections, there is always a window period for testing when only the antigensare tested [9].

A new parameter was introduced for diagnosing a dengue infection called the NS1 antigen. It was found positive detected from the first day of fever in primary and secondary infections. Hence, it is important to note that NS1 has been shown to be highly specific viral marker, making it a very reliable parameter for diagnosing dengue infection from the first day of fever.

Thrombocytopenia is found to be an early indicator of patients with dengue infection. It plays a critical role in early identification and early treatment. However, thrombocytopenia can also be found in other viral infections, collagen vascular diseases and drug induced posing a diagnostic dilemma. But there are some valid studies done in the past to prove the importance of thrombocytopenia and platelet indices.

In a study conducted by Joshi AA et al, platelet indices are the focus of attention because of its usefulness in dengue fever. Mean platelet value (MPV) is an indicator of platelet size and is expressed as the ratio of the critical value of platelets to the total number of platelets in a hematology analyser [10].

In dengue fever, IgM levels increase after 3-5 days of fever and IgG levels increase after 6-15 days. Low platelet count, low MPV, low PCT and high PDW showed significant sensitivity and specificity for dengue and could be used as predictors for the severity of dengue infection [10].

A few research studies claim that an atypical lymphocyte count of > 10% is a good indicator of dengue infection (DF), whereas a count of > 15% has been suggested as a lab standard for dengue hemorrhagic fever (DHF). The modification in atypical lymphocyte is a useful marker to assess the severity of the infection. The pathogenesis of atypical lymphocytes is not well established. B-cell oncogenesis is the most proposed theory, contrary to the general expectation that T-cells could be the source of these cells [11].

As the disease becomes severe, there is an increase in atypical lymphocytes in dengue and may reflect an enhanced immune response with increased production of immunoglobulin (Ig) to control the spread of dengue virus-infected cells in response to viral antigens. The origin of atypical lymphocytes is uncertain. B-cell ontogenesis was proposed as the origin, contrary to the general expectation that T-cells could be the source of these cells [12].

Also observed that in some viral infectious diseases with atypical lymphocytosis, such as Ebstein Barr virus (EBV), cytomegalovirus (CMV), dengue virus, and hantavirus infection, patients often present with leucocytosis [13].

In this study, we observed that atypical lymphocytes were seen in the peripheral blood of all patients with severe fever with and thrombocytopenia syndrome during acute phase. Although only a few case reports so far describe the occurrence of atypical lymphocytes in the peripheral blood of patients, based on our observations, atypical lymphocytes appeared

more frequently in the peripheral blood of patients with severefever with thrombocytopenia syndrome.

Conclusion:

Atypical Lymphocyte Count is a reactive lymphocyte that place a vital role in the immune response. To our knowledge, there are only very few studies involved in the investigation of correlation between Platelet indices and Atypical Lymphocyte Count in Dengue cases. It was observed that as the severity of thrombocytopenia increases, Atypical Lymphocyte Count increases. This may be useful in peripheral centres where viral isolation and genomic sequencing is not possible. It helps to alert the clinician in preventing the complications and mortalities of Dengue infection.

References:

1. Yadav SNS. A study of abnormal haematological parameters in dengue fever. IAIM 2018: 5(5); 117-120
2. A Manoharan, Sinha P. Role of Mean platelet volume in Dengue fever - A prognostic marker. International journal of Research & Review 2017: Vo14; Issue 6.
3. Sharma Kritika, Yadav Ajay: Association of mean platelet volume with severity, serology and Treatment outcome in Dengue fever. Journal of clinical and diagnostic research: 2015: 9(11)
4. Tantanate C, Kilnbua C. Performance Evaluation of High Fluorescence Lymphocyte Count: Comparability to Atypical Lymphocyte and Clinical Significance. Laboratory Medicine.2018: 49(4); 362-368.
5. Clorie HSC, Abeysuriya V , Thilakawasdang UB. Atypical lymphocyte count correlates with the severity of dengue infection. PLOS one. 2019; 14(5).
6. Adewoyin AS. Peripheral blood film- A review Annals of lbadan postgraduate medicine 2014: 12(2);71-99.
7. Mukker P, Kiran S. Platelets indices evaluation with dengue fever. International journal of research in medical sciences. 2018;6(6):2054.
8. Shetty A, Kasukurti P., The reactive lymphocyte: A morphological indicator of platelet counts in dengue seropositive patients. Annals of pathology and laboratory medicine.2016;3(3).
9. Kulkarni RD, Patil SS, Ajantha GS, Upadhya AK, Kalabhavi AS, Shubhada RM, Shetty PC, Jain PA. Association of platelet count and serological markers of dengue infection importance of NS1 antigen. Indian journal of Medical Microbiology.2011;29(4):359-362.
10. Joshi AA, Tanjore R., Mean platelet volume: Has it the capacity to predict the concentration of platelet counts in dengue: A study. International journal of pathology and oncology.2020;7(3):352-356.
11. Joshi AA, Gayathri BR, Kulkarni S. Correlation of thrombocytopenia with degree of atypical lymphocytosis as a prognostic indicator in dengue. International Journal of Research Medical Science. 2017; 5(9): 4041-4043.
12. Abeysuriya V, Shalindi M. The atypical lymphocyte count: A novel predictive factor for severe thrombocytopenia related to dengue. Transactions of the Royal society of Tropical medicine & Hygienic 2020: (1416); 424-432
13. Takahashi T, Suzuki T, Hiroshige S, Nouno S, Matsumura T., Transient appearance of plasmablasts in the peripheral blood of Japanese patients with severe fever with thrombocytopenia syndrome. The journal of infectious disease. 2019; 220(1): 23-27.