

Morphological Spectrum of Lymphocytes in Dengue Seropositive Patient: A Tertiary Hospital based Study

Kabul Haque¹, Ronald RK², Arambam Gautam³, Huidrom Jyotsna⁴, Sushila L⁵

¹PGT, Department of Pathology, JNIMS, Imphal

²Senior Resident, MD, Department of Pathology, JNIMS, Imphal

³ MD, DM Haematopathologist, Department of Pathology, JNIMS, Imphal
Corresponding author Email: [gautamarambam\[at\]gmail.com](mailto:gautamarambam[at]gmail.com)

⁴Senior Resident, MD, Department of Pathology, JNIMS, Imphal

⁵Professor of Pathology, JNIMS, Imphal

Abstract: Dengue is a vector borne disease transmitted by the bite of *Aedes mosquito*. The causative organism is a flavivirus, a single stranded RNA virus. It is also called Break bone disease because of the severe muscle spasm and pain associated with the disease. The severe manifestations of the disease is dengue haemorrhagic fever. Dengue infection is diagnosed usually by serological testing for NS1 Antigen, IgM and IgG. Haematological manifestations produced by dengue infection includes low platelet count and low WBC count. Severe dengue resulting to thrombocytopenia may lead to low haemoglobin level due to bleeding. In this study, the different morphology of the lymphocytes were analysed in dengue seropositive sample in relation to severity of thrombocytopenia and WBC count. Results: A total of 103 seropositive dengue samples were analysed, 55 were males and 48 were females with the median age of 42 years. The median platelet count was 1,08,735/cu.mm with the lowest platelet count of 11000/cu.mm. The lowest haemoglobin observed was 8.2 g/dl and the maximum Haemoglobin observed was 18.8 g/dl. Transformed lymphocytes were significantly increased in those samples with low platelet count and low WBC count. **Conclusion:** Transformed lymphocytes were significantly increased in those samples with low platelet count and low WBC count. Platelet count though are reduced in dengue seropositive patient but the presence of transformed lymphocytes were significantly associated with low platelet count and low WBC count.

Keywords: Dengue, seropositive, thrombocytopenia, transformed lymphocytes, low wbc

1. Introduction

Dengue is a viral illness caused by flavivirus virus, a single stranded RNA virus transmitted by the bite of *Aedes mosquitos species*(1). It produces severe muscle pain and spasm, so it is also called breakbone fever and seventh day fever because of the usual duration of the symptoms(2). Haematological manifestations due to dengue virus infection includes low platelet count and low WBC count(3). It is usually diagnosed by serological testing for NS1 Ag, IgM and IgG immunoglobulins (4). Dengue haemorrhagic fever is the severe manifestation of Dengue infection which includes severe thrombocytopenia and sometimes bleeding (5). Most of the patients have normal haemoglobin level. Anaemia resulting due to bleeding from severe thrombocytopenia may occur in Dengue. Transformed lymphocytes are usually encountered in dengue viral infection in peripheral blood smears(6).

Aims and Objectives

To analyse the different lymphocytes morphology in relation to WBC count and platelet count.

2. Materials and Method

Cross sectional observational study done between October 2023 to November 2023 in the department of Pathology, JNIMS, Imphal. A total of 103 CBC samples of dengue

seropositive patients received from OPD as well as from the Ward were analysed in automated haematology 5 part differential analyser; erba (transasia) H560. Peripheral blood smears were made and Leishman's stains were done. The different morphology of lymphocytes were analysed in relation to WBC count and Platelet count. **Statistical analysis:** SPSS version 25 and Pearson correlation coefficient were used for statistical analysis. A P value of <0.05 were considered significant in this study. **Ethical Justifications:** This study doesn't entail adverse effect on the patients and this study does not entail any therapeutic interventions or use of experimental animals. The results were informed to the clinical team. Research was carried accordingly to institutional ethical guidelines.

3. Results and Observation

A total of 103 CBC samples of dengue seropositive patients were analysed in the department of pathology, JNIMS, Imphal. 55 were males and 48 were females. The median age was 42 years with the maximum age of 79 years. The median platelet count observed was 108735/cu.mm with the lowest platelet count of 11000/cu.mm. The lowest haemoglobin observed was 8.2 g/dl and the maximum haemoglobin was 18.8 g/dl. 73.79% seropositive samples were thrombocytopenic with the platelet count below 150000/cu.mm as shown in Fig:1. 25.24% had normal platelet count. One patient (0.97%) had platelet count more

than 450000/cu.mm. The median WBC count was 4000/cu.mm with the minimum and maximum WBC of 1440/cumm and 15560/cu.mm respectively. 53.40% were leucopenic with the WBC count less than 4000/cu.mm, 42.72% were within normal range and 3.88% dengue seropositive samples were having leucocytosis as shown in fig 2. Table 1: shows the significant correlation between platelet count and WBC count in this study with the p value of 0.02. Fig 3 highlight the different lymphocyte morphology seen in dengue seropositive samples in this study. The transformed lymphocytes were significantly elevated in those samples with low WBC and low platelet count. Plasmacytoid lymphocytes were significantly associated with those samples with low platelet and low WBC count. Plasmacytoid lymphocytes are significantly associated with those samples with low platelet count. The various transformed lymphocytes encountered in dengue sero positive samples are depicted in fig 3 and fig 4. The presence of transformed lymphocytes particularly blastoid and plasmacytoid morphology were associated with those samples with low WBC and low platelet count as shown in scatter plots (Fig 5 and 6)

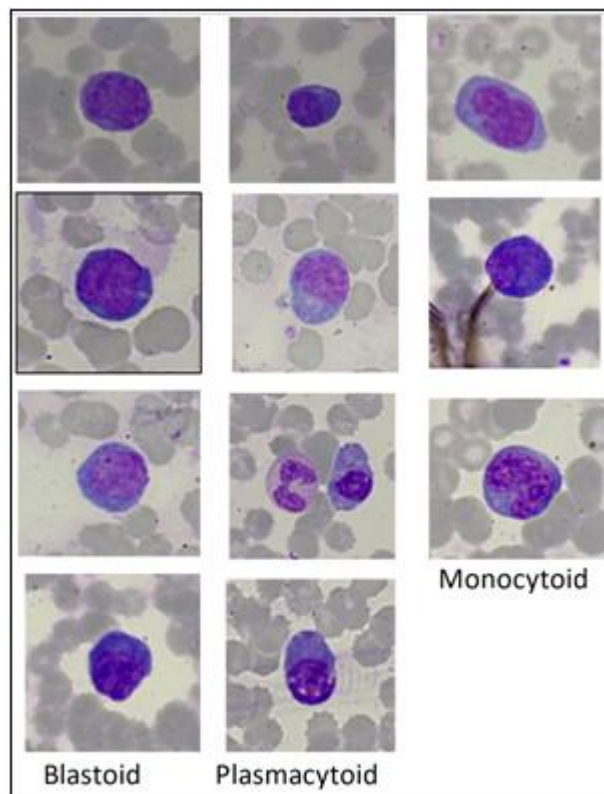


Figure 3: Leishman's stain 100x: Transformed lymphocytes in dengue seropositive samples. Blastoid, plasmacytoid and monocytoid forms

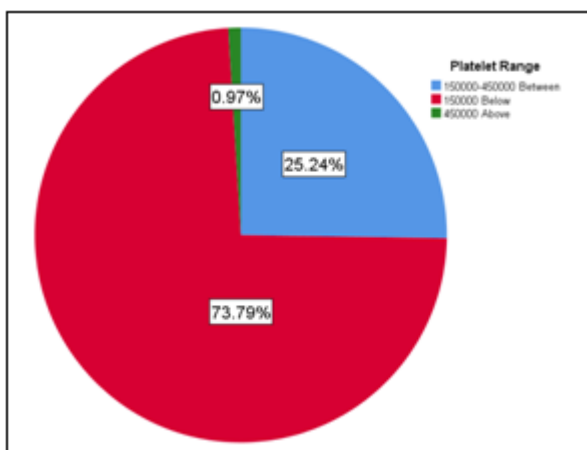


Figure 1: Pie chart of Platelet count range in dengue seropositive patients

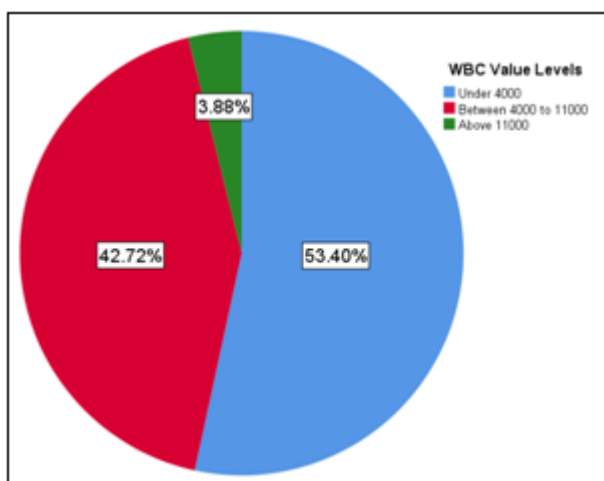


Figure 2: Pie chart of WBC distribution in dengue seropositive patients

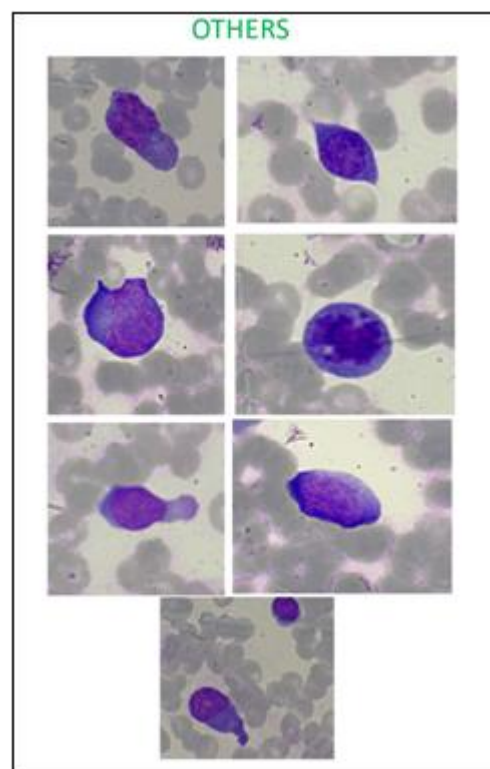


Figure 4: Transformed lymphocytes (hand mirror form, mummified form, bipolar form and with dark basophilic peripheral cytoplasmic accentuation).

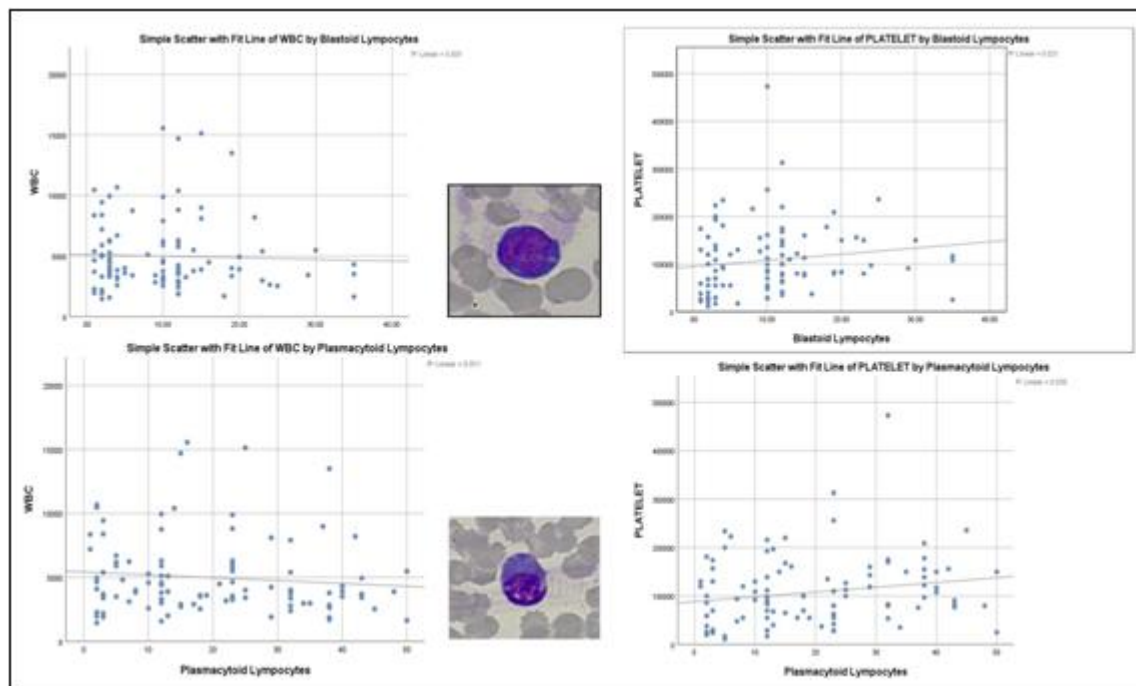


Figure 5: Scatter plot of blastoid lymphocytes, plasmacytoid lymphocytes with platelet count and WBC count

4. Discussion

The presence of blastoid and plasmacytoid morphology of lymphocytes in the peripheral blood smear were associated with low platelet count and WBC count in our study. The increased proportion of plasmacytoid lymphocytes were associated with low platelet and low WBC count in other study (7). Rubella and dengue are the disease which are associated with increased proportion of plasmacytoid lymphocytes in peripheral blood(7). Dengue can be suspected when there is thrombocytopenia, leucopenia with the increase proportion of blastoid and plasmacytoid lymphocytes on peripheral blood examination which is an easy and very cost effective test. The presence of blastoid form of transformed lymphocytes in peripheral blood may mimic blasts for the inexperienced observers. Most of the transformed lymphocytes seen in dengue seropositive samples in this study have deeply agranular basophilic cytoplasm. Reactive lymphocytes have prominent dark-blue cytoplasm that results from increased protein synthesis and increased mRNA in the cytoplasm. The nucleus may undergo blast transformation and have a convoluted shape.(8). In our study, flow cytometry was performed for one sample with increased proportion of plasmacytoid lymphocytes which were CD19 positive (B cells) positive.

5. Conclusion

Dengue is a viral infection where there is presence of transformed lymphocytes on peripheral blood examination. Transformed lymphocytes may mimic blasts by the inexperienced observers. Presence of **blastoid** and **plasmacytoid** morphology of lymphocytes in dengue is associated with leucopenia and thrombocytopenia. The plasmacytoid lymphocytes encountered in dengue in dengue are mostly B lymphocytes. Trial of immunosuppressant may be try in the management of dengue infection.

Funding: No grant/ fund received

Conflict of interest: None

Authors contributions:

- 1) Dr. Kabul Haque wrote the manuscript
- 2) Dr.ArambamGautam andDr.L. Sushila Devi contributed in design and analysis the study
- 3) Dr.Huidrom Jyotsna and Dr. R.K. Ronald collected the data

References

- [1] Hasan S, Jamdar SF, Alalawi M, Al Ageel Al Beaiji SM. Dengue virus: A global human threat: Review of literature. *J Int Soc Prev Community Dent.* 2016;6(1):1–6.
- [2] Schaefer TJ, Panda PK, Wolford RW. Dengue Fever. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 [cited 2023 Dec 11]. Available from: <http://www.ncbi.nlm.nih.gov/books/NBK430732/>
- [3] Jayanthi HK, Tulasi SK. Correlation study between platelet count, leukocyte count, nonhemorrhagic complications, and duration of hospital stay in dengue fever with thrombocytopenia. *J Fam Med Prim Care.* 2016;5(1):120–3.
- [4] EcheGARAY F, Laing P, Hernandez S, Marquez S, Harris A, Laing I, et al. Adapting Rapid Diagnostic Tests to Detect Historical Dengue Virus Infections. *Front Immunol* [Internet]. 2021 [cited 2023 Dec 11];12. Available from: <https://www.frontiersin.org/articles/10.3389/fimmu.2021.703887>
- [5] Rigau-Pérez JG, Clark GG, Gubler DJ, Reiter P, Sanders EJ, Vorndam AV. Dengue and dengue haemorrhagic fever. *The Lancet.* 1998 Sep 19;352(9132):971–7.

- [6] Clarice CSH, Abeysuriya V, De Mel S, Uvindu Thilakawardana B, De Mel P, De Mel C, et al. Atypical lymphocyte count correlates with the severity of dengue infection. Cox D, editor. PLOS ONE. 2019 May 1;14(5):e0215061.
- [7] Datta L, Menon MP. Plasmacytoid lymphocytes: a clue to dengue diagnosis. Blood. 2017 Apr 13;129(15):2202–2202.
- [8] Weiss D, Tvedten H. Chapter 2 - The Complete Blood Count and Bone Marrow Examination: General Comments and Selected Techniques. In: Willard MD, Tvedten H, editors. Small Animal Clinical Diagnosis by Laboratory Methods (Fourth Edition) [Internet]. Saint Louis: W.B. Saunders; 2004 [cited 2023 Dec 12]. p. 14–37. Available from: <https://www.sciencedirect.com/science/article/pii/B0721689035500069>