International Journal of Medical and Pharmaceutical Research

Online ISSN-2958-3683 | Print ISSN-2958-3675
Frequency: Bi-Monthly
Available online on: https://ijmpr.in/

Original Article

Lymphocyte Subset Analysis by Flow Cytometry in Dengue Patients

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Received: 23-02-2026
Accepted: 13-03-2026
Available online: 31-03-2026

ABSTRACT

Introduction- Symptoms of the mosquito-borne virus dengue may range from a low-grade fever to more serious complications including dengue shock condition and dengue hemorrhagic fever. Disease severity is largely influenced by host immune responses. T-lymphocyte subsets, particularly $CD4^+$ and $CD8^+$ cells, play an significant role in dengue immunopathogenesis, and their alterations may reflect immune dysregulation. Flow cytometry enables accurate enumeration of lymphocyte subsets and helps assess immune changes in dengue infection.
Aim-To enumerate $CD4^+$ and $CD8^+$ lymphocyte counts in dengue-infected patients using flow cytometry, standardize the enumeration method on a flow cytometry platform, and correlate $CD4^+$ and $CD8^+$ counts with the clinical severity of dengue, including uncomplicated and complicated cases.
Materials and Methods- The research was conducted at a tertiary care clinic including 35 laboratory-confirmed dengue cases (and 9 controls= total 44 cases) diagnosed by NS1 antigen and/or IgM antibody testing. Dengue cases were categorized into uncomplicated and complicated dengue based on clinical features and WHO criteria. Peripheral blood samples were composed in EDTA vacutainers as well as processed within six hours. $CD4^+$ and $CD8^+$ lymphocyte enumeration was performed using standardized protocols on a BD FACS Canto II flow cytometer. Statistical analysis was carried out with SPSS software.
Results-Age distribution among study groups was comparable. Dengue patients showed alterations in $CD4^+$ and $CD8^+$ lymphocyte counts compared with healthy controls. Patients with complicated dengue demonstrated greater deviations in lymphocyte subsets, indicating more pronounced immune dysregulation associated with disease severity.
Conclusion- Flow cytometric analysis of $CD4^+$ and $CD8^+$ lymphocyte subsets provides valuable insights into immune changes in dengue infection. Differences between uncomplicated and complicated dengue highlight the role of T-cell dysregulation in disease severity. Lymphocyte subset analysis may serve as a useful adjunct in evaluating disease progression and immune pathogenesis in dengue.

Keywords*: Dengue, Lymphocyte Subsets, CD4, CD8, Flow Cytometry, Dengue Severity.*

INTRODUCTION

The mosquito-borne virus known as dengue may cause a wide variety of symptoms in humans, from asymptomatic as well as mild dengue fever to more severe containing dengue hemorrhagic fever as well as dengue shock set of symptoms, the latter of which is marked by plasma leakage, coagulopathy, and the involvement of multiple organs. The diversity of clinical manifestation is widely explained by the multifaceted nature of the interaction of viral replication, host immune response, and inflammatory mediators. Lymphocytes are among the crucial elements of the immune system in protecting immunity as well as immunopathology that is accompanied by a severe disease. Change in lymphocyte subsets and their activation status will give us valuable information on the immunodysregulation in the case of dengue infection. [1,2]

Dengue infection is marked by major changes in innate and adaptive immunity. Lymphopenia is a general hematological result, which is thought to be caused by apoptosis, bone marrow suppression and redistribution of lymphocytes to the tissues. Flow cytometry makes it possible to quantify predominant lymphocyte populations, containing T cells, B cells, as well as natural killer (NK) cells as well as their subpopulations, containing CD4+ helper T cells, CD8+ cytotoxic T cells, controlling T cells and activated phenotypes, with precision. These subsets have different roles in the immune response; the role of CD8+ T cells is to destroy virus-infected cells, whereas the role of CD4+ T cells is to control the cellular as well as humoral immune system. Uncontrolled activation of these responses, however, can also cause systemic inflammation and vascular permeability, both of which are terminal features of severe dengue. [2,3]

One of the most evident immunological characteristics of the dengue infection is the proliferation as well as stimulation of CD8+ T cells which release cytokines like interferon- γ and tumor necrosis factor- α which aid the control of the viral replication process but can also lead to inflammatory tissue damage. The acute phase is characterized by increased appearance of activation symbols on CD4+ and CD8+ T cells (CD38 and HLA-DR), which is an indicator of increased immune activation. Shifts in the CD4+/CD8+ ratio were also attributed to the severity of the disease. [3,4]

The B lymphocytes undergo even more drastic changes in case of the dengue infection, with the radical increase of the plasmablasts growth during the acute phase. The markers of these antibody-secreting cells include CD27 and CD38 and may make a large percentage of the circulating lymphocytes in severe dengue. Although this response is important in the production of antibodies, it can also be involved in antibody-dependent enhancement in case of secondary illness. [4,5]

Regulatory T cells and natural killer cells are also convoluted in the immunological reply to dengue contagion. The changes in the number as well as activity of NK, regulating T-cell activity may also serve to promote the inability to regulate the virus and overexpression of inflammation. The flow cytometric analysis of the markers like CD56, CD16, CD25, and FOXP3 allows specifying these immune changes in detail and understanding the mechanisms of severe diseases. [5,6]

Besides surface marker results, flow cytometry may also assess functional elements of cytokine production, apoptosis and lymphocyte proliferation using such markers as annexinV and Ki-67. These parameters will give valuable information on immune activation and exhaustion in dengue infection. Furthermore, lymphocyte subset profiling may assist in differentiating dengue from other febrile illnesses and may have potential prognostic value in predicting disease severity. [6–9] The study was showed to evaluate lymphocyte subset analysis through flow cytometry in patients with dengue contagion.

MATERIALS AND METHODS

The study was showed in the Division of Pathology at B.L.D.E. (Deemed to be University), Shri B.M. Patil Medical College, Hospital and Research Centre, Vijayapura, Karnataka from March 2024 to October 2025. Total 44 cases out of which 35 confirmed dengue cases were taken into consideration for the study. Ethical permission be there obtained from the Institutional Ethics Committee (IEC) earlier beginning the research (IEC number-125/2023-24).

Inclusion Criteria

Both adult and pediatric patients with confirmed dengue. Confirmation was based on a confident test for either the Dengue NS1 antigen as well as/or Dengue IgM antibody.

Exclusion Criteria

- Patients tested positive only for Dengue IgG, Individuals with known immunosuppressive conditions (e.g., HIV, ongoing chemotherapy, chronic steroid use).
- Based on the clinical and laboratory summary, the participants were systematically categorized into three distinct study groups to enable comparative analysis:

Group 1- Uncomplicated Dengue (Mild Dengue): 17 individuals who were progressive for NS1 as well as/or IgM be present included in this group, had a mild clinical course, did not show any of the warning signs as well as also did not have any plasma leakage or severe bleeding.

Group 2- Complicated Dengue (Severe Dengue): This population was the patients who had severe immunopathological manifestations. It involved 18 patients who were NS1 and/or IgM positive and diagnosed by dengue hemorrhagic fever (DHF) or dengue shock condition (DSS) and had warning signs and/or with severe clinical manifestations.

Group 3 (Healthy Controls): This group served as the baseline control and consisted of 9 healthy individuals with no evidence of dengue infection or other acute illnesses. They provided reference values for normal CD4 and CD8 lymphocyte counts measured by flow cytometry in the local population.

The study procedure was conducted in a systematic way aimed at securing precision and reproducibility. First, the enumeration of CD4 and CD8 lymphocytes was standardized on the BD FACS-Canto II flow cytometer with the help of reference samples to define the protocol parameters such as voltage, compensation, and gating. Aseptic collection of 2 ml of peripheral venous blood from each participant existed done in an EDTA vacutainer. Within a 6-hour time- frame of collection, a 50 µL volume of whole blood was stained by 20 µL of the fluorescently-labeled monoclonal antibodies: CD4-Fluorescein Isothiocyanate(FITC) and CD8-Phycoerythrin (PE). The stained samples underwent incubation in the dusky at room temperature for 15 minutes afterward which 450 µL of 1X BD FACS lysing was used to lyse the red blood cells. Again, samples underwent incubation in the dark at room temperature for 15 minutes. The samples were then washed before being acquired on the flow cytometer. CD3+ T lymphocytes were gated, and further analysis of CD4+ and CD8+ subsets was performed within the CD3+ population. At least 10,000 events selected from the lymphocyte gate, according to forward and side scatter characteristics, were collected for every sample. The analysis was conducted using the software of the instrument, which reflected the percentages of CD4+ and CD8+ cells, and also provided the absolute counts.

Data was collected through a structured proforma. Details were entered into Microsoft Excel as well as examined with the SPSS version 28.0. A p-value of less than 0.05 was regarded as statistically substantial.

RESULT

Table 1. 'Age-wise Distribution of Study Participants Across Groups'

	Group			**Total**	**P value**
Age (Year)	**Controls**	**Mild**	**Severe**		
1 to 10	0	3	2	5	
11 to 20	1	2	6	9	
21 to 30	4	6	9	19	
31 to 40	3	3	0	6	
41 to 50	1	1	0	2	
> 50	0	2	1	3	0.238
Total	9	17	18	44	

The largest number of cases were in the age cohort of 21-30 years (43.2%) shadowed by 11-20 years (20.4%). The young adults (21-30 years) showed 50% of total severe cases, while the mild ones were more evenly distributed between younger and middle-aged ones.

Table 2. Gender-'wise Distribution of Study Participants Across Groups'

Gender	**Group**			**Total**	**P value**
	CONTROLS	**MILD**	**SEVERE**		
Female	3	10	9	22	0.465
Male	6	7	9	22	
Total	9	17	18	44	

Males and females were equally represented with each contributing 22 participants (50% of total sample). The proportion of females in mild dengue was slightly higher (58.8%), while in severe cases the dispersal was equal among males and females (50% each).

Table 3. NS1 Antigen Positivity Across Controls, Mild Dengue, and Severe Dengue Groups

	Group			**Total**	**P value**
NS1 Antigen	**Controls**	**Mild**	**Severe**		
Negative	9	10	7	26	0.010
Positive	0	7	11	18	
Total	9	17	18	44	

NS1 antigen showed a significant association with dengue severity (p = 0.010). Control was all NS1-negative with an increasing positivity with the severity of the disease (41.2% in mild disease, 61.1% in severe disease). This tendency

implies an increase in viral antigen levels during severe dengue, and NS1 is a convenient and early diagnostic and possibly prognostic value.

Table 4. IgM Seropositivity Across Controls, Mild Dengue, and Severe Dengue Groups

	Group			**Total**	**P value**
Ig M	**Controls**	**Mild**	**Severe**		
Negative	9	6	5	20	0.001
Positive	0	11	13	24	
Total	9	17	18	44	

There was a very significant association between dengue status and severity with IgM positivity (p = 0.001). The test was specific as all the control participants were negative on IgM. The percentage of IgM-positive persons in the mild and severe cases of dengue was 64.7 and 72.2 respectively, which represents active or recent infection. The growing ratio of IgM positivity between mild and severe cases of dengue is an indicator of augmented humoral reaction alongside the onset of the illness.

Table 5. Comparison of TLC, Platelet Count, and Lymphocyte Subsets Across Education Groups

		N	Mean	Std. Deviation	P value
Total Leucocyte Count (103/µL)	CONTROLS	9	7.0100	1.58682	0.504
	MILD	17	7.2524	3.43300	
	SEVERE	18	8.8694	6.44407	
	Total	44	7.8643	4.68985	
Platelet count(103/µL)	CONTROLS	9	251.67	63.806	
	MILD	17	241.71	86.341	
	SEVERE	18	100.83	71.701	0.000
	Total	44	186.11	103.583	
CD 3 Absolute count (cells/µL)	CONTROLS	9	1407.89	315.650	
	MILD	17	1160.47	628.930	
	SEVERE	18	943.56	803.449	
	Total	44	1122.34	672.272	0.033
CD 4 Absolute count (cells/µL)	CONTROLS	9	724.11	190.702	0.038
	MILD	17	633.41	388.896	
	SEVERE	18	444.00	411.476	
	Total	44	574.48	378.372	
CD 8 Absolute count (cells/µL)	CONTROLS	9	602.67	173.484	
	MILD	17	435.41	238.817	
	SEVERE	18	431.06	373.059	
	Total	44	467.84	294.330	0.012
CD4/8 Ratio	CONTROLS	9	1.2433	0.36222	0.021
	MILD	17	1.5394	0.55834	
	SEVERE	18	1.1956	0.50963	
	Total	44	1.3382	0.51913	

Total leukocyte count (TLC) showed no significant difference between groups (p = 0.504), which means that it is not valuable in the measurement of the sickness severity. The number of platelets was found to be reduced with the severity (p < 0.001), severe dengue exhibited pronounced thrombocytopenia (100.83 ± 71.70 ×10 3/ uL). Here was also a significant reduction in absolute counts of CD3, CD4, as well as CD8 (p = 0.033, 0.038, and 0.012) indicating an effect of progressive depletion in T-cells. There was a significant variance in the CD4/CD8 ratio (p = 0.021), indicating the variability of the immune dys-regulations by the severity of the disease.

Table 6. Association Between NS1 Antigen Status and IgM Seropositivity

	NS1 Antigen		Total	P value
Ig M	Negative	Positive		
Negative	9	11	20	0.043
Positive	17	7	24	

Total	26	18	44	

The status of NS1 antigen and IgM seropositivity were found to be statistically associated (p = 0.043). Fifty-seven point seven percent of the NS1-negative people were also IgM negative, implicates early dengue infection or dengue absence. Conversely, 38.9% of cases with NS1 positive results were positive in IgM and this means that immune response is progressing. Also, 70.8% of IgM-positive patients were NS1 negative and this is a sign of late stages of infection when NS1 would not be detected but the IgM antibodies would increase. These results point out the value of complementary diagnosis of NS1 antigen and IgM antibodies in the various stages of dengue infection.

Table 7. Comparison of Hematological and Lymphocyte Subset Parameters Between Controls and Mild Dengue

	Group	N	Mean	Std. Deviation	P value
Total Leucocyte Count (103/µL)	CONTROLS	9	7.0100	1.58682	0.045
	MILD	17	7.2524	3.43300	
Platelet count (103/µL)	CONTROLS	9	251.67	63.806	0.014
	MILD	17	241.71	86.341	
CD 3 Absolute count (cells/ µL)	CONTROLS	9	1407.89	315.650	0.124
	MILD	17	1160.47	628.930	
CD 4 Absolute count (cells/ µL)	CONTROLS	9	724.11	190.702	0.154
	MILD	17	633.41	388.896	
CD 8 Absolute count (cells/ µL)	CONTROLS	9	602.67	173.484	0.083
	MILD	17	435.41	238.817	
CD4/8 Ratio	CONTROLS	9	1.2433	0.36222	0.143
	MILD	17	1.5394	0.55834	

The hematological parameters which included significant differences were compared in the control and the mild cases of dengue cases. TLC showed a slight difference with mild dengue and reached statistical insignificance (p = 0.045), therefore, showing that early changes in leukocytes occurred at an early stage of the infection. The reduction in platelet count in minor cases of dengue was significant (p = 0.014), therefore, demonstrating the early development of the dengue-related thrombocytopenia even in the case of patients having minor dengue. Even though absolute CD3, CD4, and CD8 counts were low in mild cases than they were in controls, the change was not statistically significant indicating that marked T-cell suppression is delayed until later or severe stages. The CD4/CD8 ratio was higher in mild dengue but not statistically significant (p = 0.143), indicating early immune activation without substantially disturbing the balance.

Table 8. Comparison of Hematological and Lymphocyte Subset Parameters Between Mild and Severe Dengue

	Group	N	Mean	Std. Deviation	P value
Total Leucocyte Count (103/µL)	Mild	17	7.2524	3.43300	0.019
	Severe	18	8.8694	6.44407	
Platelet count (103/µL)	Mild	17	241.71	86.341	0.454
	Severe	18	100.83	71.701	
CD 3 Absolute count (cells/ µL)	Mild	17	1160.47	628.930	0.824
	Severe	18	943.56	803.449	
CD 4 Absolute count (cells/ µL)	Mild	17	633.41	388.896	0.879
	Severe	18	444.00	411.476	
CD 8 Absolute count (cells/ µL)	Mild	17	435.41	238.817	0.044
	Severe	18	431.06	373.059	
CD4/8 Ratio	Mild	17	1.5394	0.55834	0.659
	Severe	18	1.1956	0.50963	

Comparison of hematological and lymphocyte subset parameters between mild and severe dengue revealed a few significant differences. In severe instances of dengue, total leukocyte count (TLC) was found to be significantly higher (p = 0.019) which shows that there is an upsurge in inflammatory response. The number of platelets was also reduced in severe dengue (100.83 71.70 ×10 3/ uL) than in mild cases (241.71 86.34 x10 3/ uL) but not significantly (p = 0.454). The absolute counts of CD3, CD4 and CD8 lymphocytes were reduced in severe dengue indicating progressive T-cell depletion and statistical significance was observed in CD8 counts (p = 0.044). The ratio of CD4/CD8 was not also significantly dissimilar among the groups (p = 0.659). In general, these results suggest that there is a decrease in T-cell subsets especially the CD8 cell as the disease severity increases in dengue infection.

Table 9. Comparison of Hematological and Lymphocyte Subset Parameters Between Controls and Severe Dengue

	Group	N	Mean	Std. Deviation	P value
Total Leucocyte Count (103/µL)	Controls	9	7.0100	1.58682	0.004
	Severe	18	8.8694	6.44407	
Platelet count (103/µL)	Controls	9	251.67	63.806	0.025
	Severe	18	100.83	71.701	
CD 3 Absolute count (cells/µL)	Controls	9	1407.89	315.650	0.007
	Severe	18	943.56	803.449	
CD 4 Absolute count (cells/µL)	Controls	9	724.11	190.702	0.044
	Severe	18	444.00	411.476	
CD 8 Absolute count (cells/µL)	Controls	9	602.67	173.484	0.034
	Severe	18	431.06	373.059	
CD4/8 Ratio	Controls	9	1.2433	0.36222	0.246
	Severe	18	1.1956	0.50963	

Comparison of controls and severe dengue patients indicated major difference in various parameters. TLC was much greater in severe dengue ($p = 0.004$), which shows a great inflammatory reaction, whereas the count of platelets was much less ($p = 0.025$), which represents dengue-related thrombocytopenia. There was significant reduction in absolute T-cell counts: CD3 ($p = 0.007$), CD4 ($p = 0.044$), and CD8 ($p = 0.034$) at severe cases; indicating that the immune system is highly suppressed. The CD4/CD8 ratio did not however show any significant difference ($p = 0.246$). In general, extreme dengue was connected with a significant hematology anomaly and severe loss of T-cell subsets.

DISCUSSION

The research was determined to test the modifications of the lymphocyte subsets, especially the counts of the CD3, CD4, and CD8 T-cells, in dengue individuals by flow cytometry and the connection of this by the severity of the illness in control, mild, and simple dengue groups. The combination of immunological, serological, and hematological parameters will help the study to make the desired emphasis on the immune patterns, which may serve as early signs of the shift to severe dengue.

In the current study, young adults were the most affected with 21-30 year age group representing 43.2% of the total cases and half of the severe cases with no statistically significant difference among groups ($p = 0.238$). This trend indicates increased exposure of the youths in endemic regions. Even though the trend is exhibited, age was not a predictive of the severity of the disease, indicating that immunological determinants and not demographic variables are the predictors of the progression of dengue to severe stages. Green et al. [10] have reported similar immune changes regardless of age and noted that there was a significant depletion of the CD4 +, CD8 +, NK, and γδ T cells in severe dengue. Other studies including those by de Matos et al. [11] and Manh et al. [12] have also shown that there is strong CD8+ T-cell responses and early effector depletion during acute infection which points to immune activation as a major factor in the harshness of disease infection as opposed to age.

The gender distribution was not significantly associated with the severity of the disease ($p = 0.465$) and the ratio of males to females was nearly equal. This is an indication that sex was not associated with susceptibility or development of severe dengue in the current cohort. The same has been observed in the past studies where it was reported that T-cell activation and depletion were not gender dependent. The experiment by Green et al. [10] and Chandele et al. [13] indicated extensive activation and useful depletion of CD8 + T cells in dengue in either sex. Similarly, Fuentes-Miranda et al. [14] showed that there is gender-independent T-cell activation suppression mediated by viruses using the inhibition of IL-2 and CD25 expression.

There was a strong correlation between the disease groups and NS1 antigen positive ($p = 0.010$), and the higher the disease becomes mild (41.2%) to severe dengue (61.1%). The increased positivity of NS1 in severe dengue indicates the increased viral antigen load and the active viral replication. This outcome is in line with the study of Green et al. [10] who noted the early loss of lymphocytes with a high level of viral antigens. Fuentes-Miranda et al. [14] have shown viral repression of T-cell activation via inhibition of NF-AT and NF-KB signaling, which might be associated by the low number of CD4 and CD8 cells in severe dengue.

Near was significant difference in the stages of IgM seropositivity among the groups ($p = 0.001$) and the levels were 64.7% in mild dengue and 72.2% in severe dengue and negative in controls. This proves IgM to be a good indicator of active or recent dengue infection but does not itself indicate the severity of the disease. The elevation of IgM levels is probably a sign of the change of early antigenemia to humoral immune response. Green et al. [10] reported similar findings with the

activation of immune in cases of acute dengue infection. Activation of T- cells impairment as defined by Fuentes-Miranda et al. [14] can contribute to increased humoral responses in severe disease.

The comparison between controls, mild, and severe dengue showed progressive changes in immunological changes according to the severity of the disease. The number of platelets dropped drastically ($p < 0.001$) which validated the dengue-related thrombocytopenia. The T-cell suppressions in severe dengue were observed in terms of reducing the number of lymphocytes in the CD3 ($p = 0.033$), CD4 ($p = 0.038$), and CD8 ($p = 0.012$) subset along the disease severity. Similar findings were conveyed by Green et al. [10], who perceived marked depletion of T-cell subsets in dengue hemorrhagic fever. Viral inhibition of T-cell activation pathways described by Fuentes-Miranda et al. [14] provides a mechanistic explanation for this lymphopenia. Additionally, de Matos et al. [11] demonstrated delayed expansion of proliferating $CD8^+$ T cells during viral clearance, while Chandele et al. [13] reported activation-induced functional exhaustion of $CD8^+$ T cells. Protective $CXCR5^+CD8^+$ T-cell reactions negatively associated with disease-causing load in the study by Qiu et al. [15], whereas Pereira et al. [16] showed that multifunctional T cells were connected with milder disease and better platelet recovery. Early reduction of effector $CD8^+$ T cells in severe dengue was also described by Manh et al. [12].

The examination of hematological and lymphocyte subset parameters between categories of mild and severe dengue illness showed the total leukocyte count (TLC) was a lot higher in the incident of severe dengue compared to the case of mild dengue with a p-value of 0.019, and this was an indication that the inflammatory response in severe cases was much more active. Platelet counts in severe dengue, although very low compared to mild dengue did not influence statistical significance ($p = 0.454$), probably for of the high variances in the timing of platelet nadir. However, the steep drop in platelet numbers strongly suggests classical thrombocytopenia which is typically associated with severe dengue. The mean CD3+ T cell counts were lower in severe dengue as associated to mild dengue but the variance was not significant statistically ($p = 0.824$). There was also a significant decrease in CD4+ T cell count from mild to severe dengue ($p = 0.879$). CD8+ T cell was the only lymphocyte subset that showed a difference which was statistically significant ($p = 0.044$). Even though the means look very similar, the large variance depicts heavy CD8+ T cell suppression in a lot of severe cases. The $CD4^+/CD8^+$ ratio was still comparable ($p = 0.659$) which means that the suppression of different lymphocyte subsets was equal. Manh et al. described the early depletion of effector $CD8^+$ T cells in severe dengue, followed by a rebound after the fever. The severe cases in the current study probably signals the pre-rebound phase, which explains the extremely low counts. Qiu et al. [15] revealed that protective $CXCR5^+CD8^+$ T cells appeared at a later stage and had a damaging correlation with the viral load. The insufficient rise of this subset could lead to the lower $CD8^+$ counts seen in severe dengue. Sánchez-Vargas et al. [17] pointed out the hyperinflammation linked to IL-17 in DHF, which caused lymphocyte apoptosis and severe immunopathology.

Implications- This research has linked the basic research work, up to patient management, clinical, diagnostic, and public health implications that are very essential, and this implies that the findings will be utilized extensively. The profound declinations of the CD3+, CD4+, CD8+ T- cell subsets in patients with dengue indicated cellular immunity suppressions were a essential element in the pathogenesis of the syndrome and lymphocyte profiling its niche in clinical execution. Flow cytometry can be used to diagnose dengue, and it is possible that it enables clinicians to identify patients with a higher risk of developing complications before the obvious indicators appear. The implications of the findings can be also applied to the improvement of the Dengue vaccine, as the resistant patterns that have been pointed out in the study, suggest that there are some T-cell subsets that are most likely to be involved in the results, be it protective or pathological. Data collection of the study may also help to fine-tune the severity scoring systems and eventually simplify the adoption of the newly developed guidelines to dengue management of the severity criteria

Strengths and limitations- The strengths of the research include the fact that it utilizes flow cytometry-based lymphocyte subset analysis which has been used to thoroughly evaluate the immune changes in patients with dengue. Also, the use of healthy controls and comparison between mild and severe dengue groups assisted in interpreting the immunological alteration with the disease severity. The limitation of the study is that it did not take into consideration cytokine-level, regulatory T-cells, NK-cells subsets, and memory phenotypes, which may be of significant interest in determining the severity of dengue and would have been informative in addition to lymphocyte subsets analysis.

CONCLUSION

Finally, there was a substantial depletion of lymphocytes (CD3, CD4 and CD8), thrombocytopenia and an increase in leukocyte count in cases of severe dengue. The findings are indicative of immune deregulation that is accompanied by inflammatory and depressed cellular immunity. Lymphocyte subset analysis can contribute to the early detection of under risk patients with severe dengue and assist in better clinical treatment and prognosis. The identified changes in the T-lymphocyte subsets present important information which could be used to develop vaccines that could be used to regulate the host immune responses to avoid severe cases of dengue.

Acknowledgement- I would like to humbly offer my hearty thanks to the Department of Pathology who have been of great guidance, encouragement and undying support all through the process of this research work. I am particularly grateful to

my guide due to his assisting help in time, priceless experience, and unwavering mentorship throughout the time. My family and friends are also a big assistance to me since they have always been encouraging, understanding, and supportive, a factor that has helped me complete this research successfully.

Conflict of Interest-'The authors declare no conflict of interest'.
Funding source -None

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