

Original Research Article

Dynamics of differential count in dengue

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ABSTRACT

Background: Dengue can occur as epidemics in India. Early diagnosis reduces mortality. Differential white cell count can aid in diagnosing and prognosticating Dengue in resource limited areas. Aim and objectives of this study were to assess patterns and utility of Differential counts in Dengue.

Methods: A total of 132 serologically positive Dengue cases were analysed over the month of November 2016. Hematology data obtained from analysers and Leishman smears were tabulated and analysed.

Results: The study showed lymphocytosis as the predominant pattern (65%) followed by neutropenia (30%), neutrophilia (11%), eosinophilia (5%), monocytosis (5%) and basophilia (4%). Atypical lymphocytosis $\geq 15\%$ were noted in 65% of the cases with 83% showing Plasmacytoid lymphocytes, 8% apoptotic lymphocytes and 43% showed other atypical lymphocytes. Also, 52% of lymphocytosis and 33% of neutrophilia cases showed severe thrombocytopenia (≤ 0.5 lakhs per cu mm). Lymphocytosis was noted to be an early event but was established in later stages as seen with serology pattern association, 28% associated with NS1 antigen test (non-structural protein 1) and 42% with antibody pattern. However, neutrophilia with 60% of cases seen in antibody pattern was a late event. Plasmacytoid lymphocytosis was noted uniformly through all serology patterns in contrast with other atypical lymphocytosis which was seen mostly (48%) in antibody pattern. Apoptotic lymphocytosis was also a late event associated mainly with antibody pattern (55%).

Conclusions: The Differential white cell count can be a useful supplementary test along with serology in resource limited peripheral areas. It additionally serves to drastically reduce morbidity and mortality.

Keywords: Atypical lymphocytes, Dengue, Differential white cell count, Lymphocytosis, Neutrophilia, Plasmacytoid lymphocytes

INTRODUCTION

Dengue fever, an arboviral infection transmitted by *Aedes* mosquito shows a range of clinical presentations from asymptomatic cases to undifferentiated fever, Dengue Hemorrhagic Fever/ Dengue Shock Syndrome or severe Dengue.¹ The disease affects 100 million worldwide with 25,000 deaths annually.² While most cases are self-limited, complications have a high morbidity and mortality if not diagnosed and treated early.³ The diagnosis in Dengue rests on clinical findings and Laboratory tests.⁴ Hematology tests (especially the

hemogram) are an important guide in therapy and prognosis.¹

The Differential count and other blood counts can supplement serology and aid diagnosis and prognosis, especially in resource limited areas.^{2,5}

This study evaluates the utility of Differential count in the Diagnosis and Prognosis of Dengue. The aim of this study is to assess; Utility of Differential count and blood counts in Dengue, Association of the Differential count with Serology and Patterns of Differential counts in Dengue.

METHODS

This is a prospective study done on 132 patients with positive Dengue serology in Hematology Department over a one month period during November 2016. All patients with serological confirmation of Dengue [NS1, IgM and IgG positivity by rapid card method (Standard diagnostics – Bioline Athena)] with results of hemogram were included in the study (done by automated Hematology Analyser – Sysmex 1800i). The peripheral smear stained by Leishman’s stain (as per hospital protocol, to verify the platelet counts) was analysed for Differential white cell count, total count and platelet count and the results were tabulated. An extended Differential count was performed on 100 white cells and the type and number of atypical lymphocytes was also tabulated. The patients with concomitant infections like Malaria and Typhoid were excluded from the study. Data of hemogram was tabulated for analysis and results of Dengue tests were retrieved from Microbiology register.

RESULTS

Analysis of a total of 132 patients positive for Dengue serology, for their differential white cell counts and other relevant hematology data was performed. The age range in this study was 5 to 65 years, with an average of 32 years with most in the 12 – 25 years age group (Table 1). There was a slight male predominance with male: female ratio of 1.2:1 (Table 1).

Hematology data

- Differential White Cell Count [reference range = 6]

Table 1: Age and sex distribution of patients.

Age group	No.	%	Gender	No.	%
≤ 12 years	35	27	Males	73	55
> 12 years	97	73	Females	59	45
Total	132	100	Total	132	100

The differential count was done and categorized into the following patterns (Table 2)

- i) Smears with Normal Differential counts
- ii) Lymphocytosis**
- iii) Neutrophilia
- iv) Eosinophilia
- v) Monocytosis
- vi) Basophilia
- vii) Neutropenia

ii and iii as the predominant pattern. iv, v, vi, vii could be overlapping with other patterns. (**Lymphocytosis includes both typical and atypical lymphocytes). The age and gender distribution showed that Lymphocytosis was uniform across the ages and both sexes (Table 3).

Table 2: Differential count pattern.

DC pattern	No. N=132	%	Range (%)	Average (%)
Normal	32	24	–	–
Neutrophilia	15	11	42 – 88	65
Lymphocytosis	85	65	46 – 88	65
Eosinophilia	07	05	07 – 12	09
Monocytosis	06	05	10 – 15	12
Basophilia	05	04	01 – 02	02
Neutropenia	40	30	10 – 44	27

Table 3: Age and gender distribution of differential counts.

DC type	Age				Gender				Total
	≤ 12 years		>12 years		Males		Females		
	No.	%	No.	%	No.	%	No.	%	
Normal	06	17	26	27	18	25	14	24	32
	23	66	62	64	45	62	40	68	85
	06	17	09	09	10	13	05	08	15
Total	35	100	97	100	73	100	59	100	132

Neutrophilia was however, noted in a higher proportion of cases of children (17%), as against adults (9%) and also seen in a slightly higher proportion of males (13%) when compared with females (8%). 74/132 Cases (56%) showed increased band forms.

The lymphocytes were categorized as (Table 4)

- i) Typical and

- ii) Atypical.⁶

Atypical includes (Table 5 and 6)

- Plasmacytoid lymphocytes-Cells with eccentric nuclei, deep blue cytoplasm with or without tailing pattern of cytoplasm and with or without increase in cell size.⁷⁻⁹

- Other atypical lymphocytes-These include lymphocytes that resemble monocytes, blasts or unclassifiable cells.
- Apoptotic lymphocytes-These include mononuclear cells with pyknotic nucleus/ nuclear fragments.

Table 4: Distribution of typical and atypical lymphocytes.

Type of lymphocytes	Number	Percent
Typical*		
≤40%	99	75
> 40%	33	25
Atypical		
≥10%	46	35
≤15%		
>15%	86	65

*Typical lymphocytes ≤40% exclude mild Dengue.

Table 5: Distribution of atypical lymphocytes.

Type of atypical lymphocytes	Total n=132		Adults n=97		Children n=35	
	No.	%	No.	%	No.	%
Plasmacytoid lymphocytes	110	83	85	88	25	71
Other atypical lymphocytes	60	45	45	46	15	43
Apoptotic lymphocytes	11	08	09	09	02	06

Table 6: Degree of atypical lymphocytosis.

Type of lymphocyte	≤5 cells - <10 cells (%)		≥10 cells (%)	
	No.	%	No.	%
Plasmacytoid lymphocytes n=110	30	27	32	29
Other atypical lymphocytes n=60	20	33	25	42

The normal range of atypical lymphocytes is between 2 – 6 %. Atypical lymphocytes >10% are a good indicator of Dengue fever.^{10,11}

An analysis of the differential count patterns with thrombocytopenia showed that lymphocytosis was associated more with severe thrombocytopenia than mild thrombocytopenia.

Table 7: Thrombocytopenia in association with differential count.

DC type	≤0.5 lakhs/cumm		>0.5 lakhs/cumm	
	No.	%	No.	%
Lymphocytosis n=85	50	59	35	41
Neutrophilia n=15	05	33	10	67
Normal pattern n=32	20	62	12	68

The reverse was true with neutrophilia, more number of cases were associated with mild thrombocytopenia than severe thrombocytopenia as noted in Table 7.

An analysis of association of total white cell count with Differential Count showed Lymphocytosis in 33% of cases with leucopenia and a minority with high counts. On the contrary neutrophilia was seen mainly with high white cell counts (Table 8).

Table 8: Total white cell count in association with differential count.

DC type	Normal TC		Decreased TC		Increased TC	
	No.	%	No.	%	No.	%
Lymphocytosis n=85	53	62	28	33	04	04
Neutrophilia n=15	04	27	05	33	06	40
Normal pattern n=32	14	44	14	44	04	12

An analysis of association of serology with differential count showed that there were higher proportion of cases of Lymphocytosis with antibody only pattern but it was present in NS1 antigen pattern also, whereas neutrophilia was significantly associated with antibody only pattern in comparison to NS1 antigen pattern (Table 9).

Table 9: Serology in association with differential count.

DC type	NS1 antigen		NS1 antigen and antibody		Antibody only	
	No.	%	No.	%	No.	%
Lymphocytosis n=85	24	28	26	30	35	42
Neutrophilia n=15	01	01	06	39	08	60
Normal	11	34	09	28	12	38

Table 10: Serology in association with significant atypical lymphocytes.

Type of atypical lymphocyte	NS ₁ antigen		NS ₁ antigen and antibody		Antibody only	
	No.	%	No.	%	No.	%
Plasmacytoid lymphocytes n=62	20	32	18	29	24	39
Other lymphocytes n=45	07	16	16	36	22	48
Apoptotic lymphocytes n=11	01	09	04	36	06	55

The analysis of serology with significant atypical lymphocytosis showed significantly higher number of apoptotic lymphocytosis associated with antibody only pattern (Table 10).

DISCUSSION

Our study showed an age range of 5 months to 65 years with an average of 32 years with a male predominance of 1.2:1 in accordance with other studies.^{1,12} It is more in males due to occupational and recreational activities.¹

An analysis of the Differential counts showed lymphocytosis in 65% (85/132) cases (lymphocyte count >45%) in accordance with few studies.^{9,13} Few studies showed lower proportion and others a higher proportion of cases.^{1,2,7}

Our analysis of lymphocytes showed atypical lymphocytosis >10% and <15% in 35% and >15% in 65% cases. A few studies give overall atypical lymphocyte count >10% with variation between 67 to 91%.^{10,13,14}

An atypical lymphocyte count of >10% is a good indicator of Dengue fever with sensitivity of 50% and specificity of 88%.^{10,11}

This study showed 83% cases with Plasmacytoid lymphocytes and 45% with other atypical lymphocytes. We could not find studies along similar lines to compare this data with. Plasmacytoid and Apoptotic lymphocytes were noted in higher proportion in adults than children.

Lymphocytosis with Plasmacytoid lymphocytes is a response to viral antigens followed by transformation to plasmacytoid lymphocytes to control the spread of infection and represents enhanced immunoglobulin production in Dengue.^{7,15} Plasmacytoid lymphocytes provide a diagnostic clue and aid in differential diagnosis of Dengue.^{7,8}

It was observed that atypical lymphocytes could serve as a marker of disease activity and are present in a higher number of cases in DHF rather than DF.^{9,11}

Severe Dengue is marked by T and B cell activation and apoptosis. Activation of 'T' cells results in release of inflammatory cytokines triggering death of cells through apoptosis which contributes to severe dengue.¹⁶

Neutrophilia was noted in 11% of cases and a normal differential in 24%. However, few studies showed a higher proportion of cases with neutrophilia.¹⁷ Neutrophilia was attributed to stress induced shift to left.¹⁷ Our study showed 56% with increased band forms in accordance with few studies.¹⁷ The demographic analysis showed higher proportion of neutrophilia in males and children.

We had 30% of cases with neutropenia in concordance with few other studies.¹⁸ However, few studies showed a higher proportion of cases.¹⁹ We didn't observe any cases of severe neutropenia but few studies had a higher proportion of severe neutropenia.¹⁹

Neutropenia is attributed to decrease in granulocytes due to bone marrow suppression in early phase and to degeneration of mature neutrophils with shift to left in febrile phase.^{13,20}

Neutrophil nadir occurs on day 4 and marks the day of defervescence. Neutrophil counts are used as an indicator of acute dengue.¹⁷

Monocytosis ($\geq 10\%$) was noted in 5% of cases in the study. Few studies had a higher proportion of cases.^{13,17} Monocytes are major targets of Dengue viruses, the entry of which into monocytes is enhanced by cross reactive antibodies resulting in increased numbers. Monocytosis along with thrombocytopenia is an early and consistent guide in diagnosis of Dengue and a marker of severe Dengue.¹⁷

Eosinophilia (>6%) was observed in 5% of the cases in this study in accordance with few studies.^{13,18} However, other studies have shown a higher proportion of cases with eosinophilia.²¹ Eosinophils decrease in acute phase but either return to normal levels or increase during convalescence and so eosinophil count can serve as a recovery parameter in Dengue fever.¹⁵

Basophilia ($\geq 1\%$) was observed in 4% cases in our study as observed in a few other studies, although some other studies have demonstrated a higher proportion of cases with basophilia.^{18,13} Basophilia indicates recovery from bone marrow suppression during convalescence along with other findings serves as a recovery parameter in Dengue.¹⁵

An analysis of total white cell counts in association with the differential counts showed 33% cases with lymphocytosis had leucopenia. Other workers showed lower proportion of cases while some others demonstrated a higher proportion of cases.^{22,9}

Neutrophilia was noted in significantly higher proportion (40%) of cases with increased rather than decreased total counts. Most cases with Lymphocytosis had normal total counts in accordance with few studies.⁹

This study observed that a higher proportion of cases of lymphocytosis (59%) was associated with severe thrombocytopenia as against 33% cases of neutrophilia indicating that lymphocytosis could perhaps serve as prognosticator of severe Dengue. An analysis of differential counts with serology patterns showed a significantly high proportion of cases of neutrophilia (60%) with antibody pattern whereas with NS1 antigen, it

was only 1% suggesting neutrophilia is late event in Dengue.

Lymphocytosis showed higher proportion of cases with antibody pattern (42%) against 28% with NS₁ antigen pattern suggesting that though lymphocytosis starts early, it is established in the later stages of the disease.

This study of the association of significant ($\geq 5\%$) atypical lymphocytosis with serology pattern noted that the appearance of plasmacytoid lymphocytes was perhaps an early event as 32% of cases were associated with NS₁ antigen and it was noted in equal number of cases (39%) in the antibody pattern whereas 48% of cases displayed other types of atypical lymphocytes in the antibody pattern with only 16% associated with NS₁ antigen.

Also, a high proportion of cases (55%) of apoptotic lymphocytes was noted with antibody pattern as against 9% with NS₁ antigen pattern suggesting that apoptosis was a late event according to a few studies which have claimed that apoptosis of infected mononuclear cells indicates improvement of clinical condition.¹⁷

Limitations of the study: These include small study size, few similar studies to compare our data with, and also that strict age related reference ranges have not been employed for the differential counts.

CONCLUSION

Dengue is a public health concern in developing and resource limited countries like India where it can occur in epidemics. Early appropriate management is crucial as currently there is no antiviral drug or vaccine available for it, and if left untreated can lead to high morbidity and mortality.

Early diagnosis is rendered by appropriate laboratory tests supplementing clinical findings. We find the Differential white cell count is one such simple, easy, cheap test of great value in resource limited areas in diagnosis and prognosis in Dengue.

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