Original Research Article

Correlation of thrombocytopenia with degree of atypical lymphocytosis as a prognostic indicator in dengue

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ABSTRACT

Background: Dengue viral infections may occur as epidemics and are of public health concern in India especially due to high mortality in advanced stages. Early diagnosis and treatment reduces the mortality significantly. To assess the utility of atypical lymphocyte counts with relation to platelet counts as a marker for severe dengue.
Methods: A total of 132 serologically proven (by rapid card method) cases of dengue with atypical lymphocytosis ($\geq$10%) and thrombocytopenia ($<150\times109$/l) were analyzed during November 2016. The complete blood counts (obtained from automated hematology analyzer and Leishman stained peripheral smears) were analyzed.
Results: Dengue infections were mostly seen in younger age (12-25 years) with slight male predominance. The relevant haematology findings noted were leukopenia in 36% and severe thrombocytopenia ($<50\times109$/l) in 57% of cases. The differential count showed lymphocytosis in 64% with significant ($\geq$20%) atypical lymphocytes in 52% which inversely correlated with platelet counts. Atypical lymphocytosis ($\geq$20%) was noted in 9% of cases with mild as against 32% of severe thrombocytopenia.
Conclusions: Atypical lymphocyte count can be used as predictor of severe thrombocytopenia and severe dengue. This helps in early diagnosis and proper management and drastic reduction in mortality.

Keywords: Atypical lymphocytes, Dengue fever, Lymphocytosis, Thrombocytopenia

INTRODUCTION

Dengue, an arboviral infection caused by one of the four serotypes of dengue viruses (DENV 1-4) is transmitted by 'Aedes' mosquito.[1,2] Clinically, it presents with headache, fever, retro-orbital pain, myalgia/body pain, rash.[3] Around 100 million cases of dengue are reported worldwide with 2,50,000 cases of Dengue hemorrhagic fever and 25,000 deaths per year.[2] In India, epidemics are becoming frequent and straining the limited resources of the public health system[4]

Dengue was previously classified as undifferentiated fever, classic dengue fever, dengue haemorrhagic fever and dengue shock syndrome.[5] Most cases of dengue are self-limiting, but mortality from complications may be around 20%, however if diagnosed early it can be reduced to 10% or less.[4] The WHO 2009 classification categorizes dengue as non-severe dengue: without and with warning signs and severe dengue to facilitate immediate and better management and surveillance.[6,7] Patients with warning signs warranted close monitoring, however these signs were based on clinical features which were nonspecific and overlapped in different diseases making it difficult to identify progression of the disease. However, in association with non-clinical predictive tools, progression to severe dengue could be identified.[8] Several studies conducted earlier emphasized the utility of lab tests especially hematology parameters in this context.[9,10] The utility of platelet counts, hematocrit and white cell counts has been explored in many studies as prognosticators in dengue.[10,11,14,15]

However, some studies only have touched upon the significance of atypical lymphocytes, their morphology and counts as predictors of severity in dengue. These studies conclude that atypical lymphocytes may play a role in predicting severity in dengue.[5,6,10,16-18]

Present study explores this line of thought by drawing parallels between the degree of atypical lymphocytosis and thrombocytopenia, and thus the progression to severe dengue; as thrombocytopenia has been associated with bleeding tendencies and increased vascular leakage with shock.[3-5,9,11,20]

Aim of this study is to assess the utility of simple, easily available tests like platelet counts and atypical lymphocytes as predictors of severity in dengue.

METHODS

This is a prospective study done on 132 patients with dengue positive serology in Hematology section of Kempegowda Institute of Medical Sciences Hospital and Research centre, Bengaluru over a one-month period in November 2016.

Inclusion criteria

All patients with serological confirmation of dengue (NS1/IgM and/or IgG positivity) done by rapid card method (standard diagnostics: Bioline Alera) with associated findings of thrombocytopenia (platelet counts <150×109/l) and atypical lymphocyte counts (≥10% of differential count) were included in the study.

Exclusion criteria

- Patients with dengue seropositivity without atypical lymphocytosis ≥10% associated with thrombocytopenia
- Patients with concomitant infections like malaria, typhoid along with dengue.

The results of dengue tests were retrieved from microbiology register. The hematological data (obtained from Automated Hematology Analyzer, Sysmex 1800i) of these cases was documented for analysis. The peripheral smear stained by Leishman's method (made for cross verification of platelet counts as per routine hospital protocol) was screened for number of atypical lymphocytes (expressed as percent in differential count) and a check of platelet counts visually were done and tabulated.

RESULTS

Demographic profile

A total of 132 dengue serology positive cases were analyzed and showed an age range between five months

to 65 years with most patients in the 12-25 years age group. The average age was 32 years (Table 1).

Table 1: Distribution of adult and pediatric age group (≤ 12 years).

Age group	Number of cases (n)	Percent (%)
*Adult >12 years	97	73
Pediatric ≤12 years	35	27

*>12 years has been categorized under adults for simplicity's sake.

There was a slight male predominance with a male to female ratio of 1.2:1 (Table 2).

Table 2: Gender wise distribution.

Gender	Number of cases (n)	Percent (%)
Males	73	55
Females	59	45

Lab data

An analysis of relevant hematology parameters was done.

Total white cell count was in the range of 1.1×109/l to 14.3×109/l with one peak at 33.4×109/l.

Leucopenia (<4.0×109/l) was observed in 36% (47 cases) and leucocytosis (>11×09 /l) in 9% (12 cases) (Table 3).

Table 3: Total white cell count distribution.

Total count (10^9/l)	Number of cases (n)	Percent (%)
<1.9	07	06
2.0-3.9	40	30
4.0-10.9	73	55
>11	12	09

An analysis of differential white cell counts showed lymphocytosis in 64% of cases (that is lymphocyte count >45% in adults and >60% in children).

The lymphocyte population showed atypical lymphocytes cells which were slightly larger, with more basophilic cytoplasm with or without abundant cytoplasm, central to eccentric nuclei, with or without conspicuous nucleoli. Some had cytoplasmic tail (Figures 1, 2, 3 and 4).[5,10,17]

The degree of atypical lymphocytosis was categorized as mild with atypical lymphocyte counts between 10-19%, moderate with counts between 20-39% and severe with counts 40-99% (Table 4).

Significant (≥20% more than moderate) atypical lymphocytosis was noted in 69 cases (52%). The highest atypical lymphocyte count was 58%.

Table 4: Degree of atypical lymphocytosis distribution.

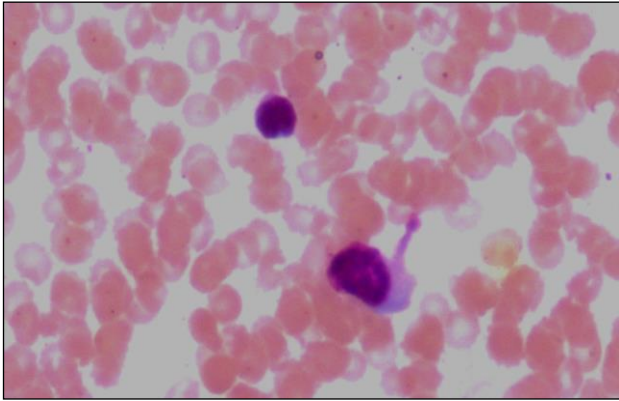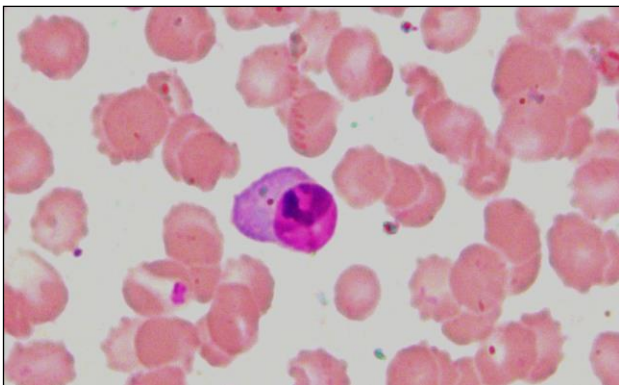| Degree of atypical lymphocytes (%)	Number of cases (n)	Percent (%)
Mild (<19%)	63	48
Moderate (<39%)	47	36
Severe (<99%)	22	16

Figure 1: Photomicrograph of a blood film. Small lymphocyte (above) and reactive lymphocyte (below) (Leishman's stain, 400x).

An analysis of thrombocytopenia (<150×109/L) showed that the minimum platelet count was 8.0 x 109/L. The degree of thrombocytopenia was graded as mild with platelet counts between 76-150×109/L, moderate with counts between 50-75×109/L and severe with <50x109/L (Table 5).

Figure 2: Photomicrograph of a blood film. Plasmacytoid lymphocyte (Leishman's stain, 1000x).
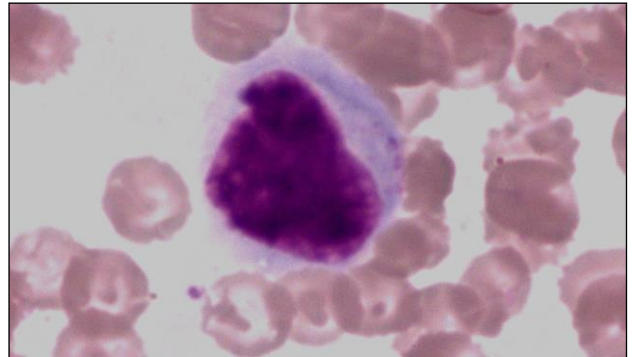

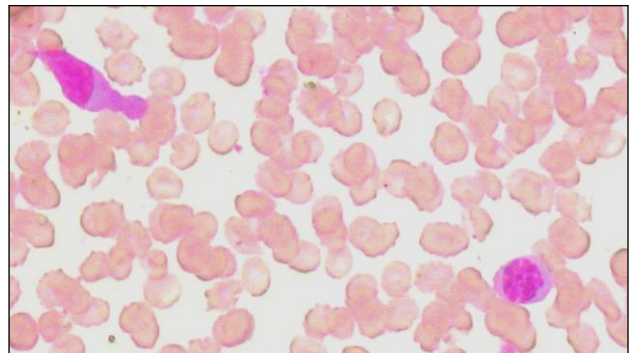**Figure 3: Photomicrograph of a blastoid lymphocyte (Leishman's stain, 1000x).**

Figure 4: Photomicrograph of a blood film. Reactive lymphocytes (Leishman's stain, 400x).

Table 5: Degree of thrombocytopenia.

Degree of thrombocytopenia (10^9/l)	Number of cases (n)	Percent (%)
Mild (<150)	30	23
Moderate (<75)	27	20
Severe (<50)	75	57

Table 6: Age wise distribution of atypical lymphocytes and platelet counts.

Age group (years)	Atypical lymphocyte count (n=69)*		Platelet count (n=75)**	
	Number of cases (n)	Percent (%)	Number of cases (n)	Percent (%)
Adults (>12 years)	48	70	54	72
Pediatric (≤12 years)	21	30	21	28

*Atypical lymphocyte counts ≥20%, **Platelet count <0.5 l/cumm

An analysis of age wise and gender wise distribution of atypical lymphocytosis (≥20%) and severe thrombocytopenia (<50×109/L) was done (Tables 6 and 7).

It was also noted that significant atypical lymphocytosis was noted in 48 of 97 (49%) adult cases as against 21of 35 (60%) pediatric cases. There was a marginal preponderance of significant atypical lymphocytosis in females (33 out of 59 or 56%) as against males (36 out of 73 or 49%).

Table 7: Gender wise distribution of atypical lymphocytes and platelet counts.

Gender	Atypical lymphocyte count (n=69)*		Platelet count (n=75)**	
	Number of cases (n)	Percent (%)	Number of cases (n)	Percent (%)
Males	36	52	43	57
Females	33	48	32	43

*Atypical lymphocyte counts ≥20%; **Platelet count <0.5 L/cumm

In case of thrombocytopenia, it was noted that 54 of 97 (56%) of adults had severe thrombocytopenia as against 21 of 35 (60%) of pediatric cases. Severe thrombocytopenia was noted in 43 of 73 males (59%) as against 32 of 59 (54%) females (Table 8).

Table 8: Correlation of atypical lymphocytes and platelet counts.

Degree of atypical lymphocyte count (%)	Platelet counts (10^9/l)					
	<50		<75		<150	
	Cases (n)	Percent (%)	Cases (n)	Percent (%)	Cases (n)	Percent (%)
Mild (<19%)	32	24	13	10	18	14
Moderate (<39%)	32	24	08	06	07	05
Severe (<99%)	11	08	06	05	05	04

Present study showed atypical lymphocyte count ranging from 10% to 58% with an average of 34%.

DISCUSSION

Severe forms of dengue are lethal if treatment is not initiated immediately. Clinical diagnosis of progression to severe dengue is difficult.[12] Lab tests especially hematocrit, thrombocytopenia, leucopenia and presence of atypical lymphocytes play a crucial role in predicting onset of severe dengue.

In present study, it was analyzed that age was in accordance with others where most cases were in the younger age group.[3,19] In the present study, most were between 12-25 years, the average age was 32 years, with a slight male preponderance similar to others probably due to occupational exposure and increased recreational activity in men.[1,10,11]

An analysis of hematology tests showed leucopenia in 36% of cases in the present study in concordance with others.[5,9] Few studies showed higher proportion and lower proportion of cases with leucopenia.[11,17,21,23]

Leucopenia is a prominent hematological change in dengue and is attributed to bone marrow suppression by the virus.[5,11] Thrombocytopenia is a characteristic finding in dengue. The present study showed severe thrombocytopenia (<50×10⁹/L) in 57% of cases in concordance with others.[1] Few studies showed a higher proportion and few a lower proportion of cases with severe thrombocytopenia.[3,17,19,22]

Thrombocytopenia is multi factorial and attributed to bone marrow suppression by antibody mediated platelet destruction, DIC and platelet injury by virus.[2,22]

The role of thrombocytopenia as prognosticator has been reported by many studies especially its association with hemorrhage manifestations and vascular leakage leading to shock.[4,11,20]

Improvements in platelet counts have been shown after therapy to indicate good prognosis.[12]

Lymphocytosis (>45% in adults and >60% in children) was noted in 64% of cases in our study in concordance with others. Few studies showed lower proportion and others higher proportion of cases with lymphocytosis.[5,11,19,23]

There is an increase in atypical lymphocytes (especially plasmacytoid type) in dengue and may reflect an augmented immune response with increased Ig production to control the spread of dengue virus infected cells5 in response to viral antigen.[5,10] The origin of atypical lymphocytes is undefined. B cell ontogeny is suggested as their origin contrary to general expectations that T cells could be the source of these cells.[16]

Present study gives the normal range of atypical lymphocytes in adults as 2-6%, being slightly higher in children.[18] A few studies claim that an atypical lymphocyte count of ≥10% is a good indicator of dengue whereas a count of ≥15% amongst others has been suggested as a lab criteria for DHF.[146,18,24] The changes in atypical lymphocyte count is a useful marker of disease activity and its concentration increases in severe cases of dengue.[6,17] It was noted that on day one of dengue fever, the counts were higher in DHF than DF and more importantly that the counts rose in tandem with a drop in platelet counts. A further association was that the atypical lymphocyte counts decrease when platelet counts recovered.[16]

Present study showed significant (≥20%) atypical lymphocytosis in 52% of cases in concordance with others, but when the cut off was ≥10%.[11,21] Few studies showed higher proportion and others showed lower proportion of atypical lymphocytosis.[10,18,23,25]

We could not get any references correlating atypical lymphocytosis (atypical lymphocyte count) and thrombocytopenia (platelet counts), perhaps it could be that ours is one of the very few studies which has attempted to explore one of the aspects suggested by few other studies about the utility of atypical lymphocyte count as a marker of disease activity and by its association with platelet counts.[6,16,17]

Present study suggests an association between degree of atypical lymphocytosis and thrombocytopenia. The atypical lymphocyte counts are inversely proportional to platelet counts with significant atypical lymphocytosis (≥20%) noted in 9% of cases with mild thrombocytopenia, but increasing to a significant 32% of cases with severe thrombocytopenia (<50×109/L) which suggests that the degree of atypical lymphocytosis could be an indicator of falling platelet counts and severe dengue.

However large-scale studies are needed to confirm these findings and check its utility as an early marker of progression to severe dengue.

Limitations of our study

- There are no standard criteria for categorization of atypical lymphocytes
- There is no cut off value for atypical lymphocytosis/degree of atypical lymphocytosis supposed to be associated with dengue
- No similar studies could be obtained for references
- The small study size is an additional limitation for accuracy.

CONCLUSION

Dengue fever, known to occur in epidemics in India is a public health concern as it has a high morbidity and mortality in its advanced stages. A combination of clinical and lab features help reduce the mortality by early recognition and management of severe dengue.

Present study is one of the few which explores the utility of a new parameter: the atypical lymphocyte count (a simple, routine test) as a predictor (possibly early marker) of severe dengue by its association with the platelet counts.

These findings however need to be confirmed by multicenter studies before the test is incorporated in the list of dengue severity predictors.

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