

Hematological Parameters in Dengue: The Serological Angle A Study

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ABSTRACT

AIM: Dengue occurs as epidemics in India. As there is no specific antiviral therapy, early diagnosis in resource poor settings helps reduce mortality. Our study is to analyse the utility of hematology parameters in association with serology patterns in dengue.

MATERIALS AND METHODS: A total of 132 serologically proven dengue cases over a month period in November 2016 along with relevant hematological data (obtained by automated hematology analyser and peripheral smears) were analyzed.

RESULTS: The age range was 5 months to 65 years with male preponderance. Serology patterns showed NS1 antigen positivity in 29% and IgG positivity in 25%. Rise in hematocrit was noted in 38% of NS1 with antibody as against 28% of NS1 only cases, indicating it was a later event. Leucopenia was prominent (87%) in NS1 (alone and NS1 with antibody) indicating it was an early marker of dengue. Leucocytosis (83%) and neutrophilia (50%) were noted in antibody patterns indicating that they could be late events (parameters). Thrombocytopenia was noted in 43% of antibody pattern as against 21% of NS1 pattern, indicating it was a late event.

CONCLUSION: In resource limited rural setups in India, haematological parameters may have utility as early markers of dengue and in association with serology aid in early diagnosis and management.

Key words: Dengue; NS1 antigen; Antibody; Blood counts

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INTRODUCTION

Dengue, an arboviral infection (DENV 1-4) transmitted by Aedes mosquito manifests as an acute febrile illness and is endemic in India. It affects 100 millions worldwide with 2,50,000 cases of dengue haemorrhagic fever and 24,000 deaths annually^[1,2]. In India, epidemics are more frequent, straining the limited resources of the public health system^[3].

The clinical manifestations of Dengue include fever, headache, muscle & joint pain, nausea, vomiting and rash. In the early phase, it has to be differentiated from other febrile illnesses like malaria, typhoid, leptospirosis etc^[4]. Dengue has been classified as Non Severe Dengue with and without warning signs and Severe Dengue (WHO 2009)^[5]. While Dengue is a self limiting condition, severe forms if not detected early and treated properly are lethal in 5-10% of

cases^[6].

Dengue is diagnosed by viral isolation, detection of viral genomic sequence by reverse transcription polymerase chain reaction (RT-PCR) (not possible in peripheral areas with limited resources in health care system) and detection of NS1 antigen with corresponding IgM, IgG antibodies by Enzyme immunoassay & Immunochromatographic test (EIA&ICT)^[1,2,7,8]. The latter are simple, rapid and easy diagnostic tests which are excellent tools for potentially fatal and epidemic prone infections^[8,9].

Apart from these, platelet count is the only lab test available in peripheral areas which supplements a diagnosis of Dengue haemorrhagic fever (DHF)/ Dengue shock syndrome (DSS)^[2,8,9]. It has been observed that a drop in platelet counts with a rise in haematocrit occurs after the 3rd day of illness. The platelet counts serve as a predictive and recovery parameter of DHF/DSS^[10].

However, it has been noted that the other hematology parameters like total white cell count, atypical lymphocyte count and haematocrit too aid in diagnosis and prognosis in dengue^[4,10,11]. While a few studies emphasised the utility of platelet counts in association with serology^[1,2]. Occasional studies only have evaluated the utility of other hematology parameters in association with serology^[12].

Our study focusses on the utility of these hematology parameters including platelet counts, but where platelet count is not an early indicator of dengue^[1], the total leucocyte counts- leucopenia & lymphocytosis are earlier and prominent events in dengue^[13] along with haematocrit in resource limited rural set ups^[14].

The aim of the study is to: (1) Assess utility of hematology parameters (total leucocyte count, differential counts, platelet counts & haematocrit) in association with serology patterns in dengue; (2) Analyse serology patterns in dengue.

MATERIALS AND METHODS

This is a prospective study done on 132 patients with positive dengue serology in hematology department of Kempegowda Institute of Medical Sciences Hospital & Research Centre, Bangalore over a one month period in Nov 2016.

The inclusion criteria were: all patients with serological confirmation of dengue (NS1, IgM, IgG antibody positivity by Rapid card method-Standard diagnostics Biolene Alera) with associated tests haematocrit, leucocyte counts, thrombocytopenia (< 1.5 lakhs/cumm) were included in the study (as low platelet counts were significant in dengue).

The exclusion criteria were: (1) Patients with dengue seropositivity with normal & increased platelet counts; (2) Patients with concomitant infections like malaria, typhoid etc along with dengue.

The haematological data (obtained from automated hematology analyser- Sysmex 1800i) was tabulated for analysis.

The peripheral smears (made for cross verification of platelet as per hospital protocol) was screened for estimation of platelets & differential counts.

Results of dengue tests were obtained from microbiology department.

Ethical committee clearance

The study involves analysis of available data for which informed consent has been obtained at the clinical side for testing.

The study maintains anonymity of patient identity by recording only age & gender against the unique hospital identification number along with relevant data.

The study was approved by ethical committee of hospital.

RESULTS

A total of 132 dengue serology positive cases were analysed. The age range was between 5 months to 65 years with most cases in the 12-25 years group. The average age was 32 years. There was a slight male predominance with male: female ratio of 1.2:1 (Table 1).

Lab Parameters

(1) Serology Test. (1) The serology pattern showed predominance of NS1 antigen followed by IgG. (2) The age & gender distribution of serology patterns showed higher proportion of NS1 antigen & antibody positive cases in adults and males compared to children & females respectively (Tables 2, 3, 4).

(2) Hematology parameters. (1) Haematocrit: A total of 72 cases with a rise in haematocrit over reference range for age & sex¹⁵ was noted in our study (Table 5). (2) Total leucocyte count: Our study showed normal counts in 73/132 cases (55%), leucopenia in 47/132 cases (36%) and leucocytosis in 12/132 cases (9%). Leucopenia is a prominent feature in dengue (Table 6 & 7). (3) Differential count: Differential count was changed in 96 of 132 cases and was normal in 36 cases (27%), 88 cases of 132 showed lymphocytosis (67%) & 8 cases (6%) showed neutrophilia. Lymphocytosis is the predominant feature begins early in disease & is established late in the disease (Table 8). (4) Platelet count (thrombocytopenia): Thrombocytopenia was categorised on the basis of platelet count as; Mild with counts < 1.5 lakhs/cumm, with 29/132 (22%); Moderate with counts < 0.75 lakhs/cumm, with 28/132 (21%) and; Severe with counts < 0.5 lakhs/cumm with 75/132 (57%). Platelet count < 1.0 lakhs/cumm is one of the defining criteria for dengue haemorrhagic fever: we had 128 cases of 132 with counts < 1.0 lakhs/cumm (Table 9).

DISCUSSION

Our study analysing age was in accordance with other studies with most cases in younger age with a slight male predominance perhaps due to occupation & recreational activities in men^[11,16].

The serology pattern showed NS1 predominance followed by IgG. Except for IgM & IgG, all patterns showed concordance with others^[17] whereas NS1, NS1 IgM patterns were similar to few studies^[1,9].

We had a lower proportion of NS1 positive pattern with a higher proportion of antibody only pattern in children as against adults. In support of this, we observed that few studies had noted a higher risk

Table 1 Age & gender distribution of platelets.

Cases	Age group		Gender		Total
	≤ 12 years	> 12 years	Male	Female	
Number	35	97	73	59	132
Percent	27	73	55	45	100

Table 2 Serology pattern in dengue.

Serology Pattern	Number	Percentage (%)
NS1 antigen	38	29
NS1 IgM	14	11
NS1 IgG	18	14
NS1 IgM, IgG	11	8
IgM	3	2
IgM, IgG	15	11
IgG	33	25
TOTAL	132	100

Table 3 Age and gender distribution of serology pattern.

Serology pattern	Adults				Pediatric			
	Males		Females		Males		Females	
	Number	Percent	Number	Percent	Number	Percent	Number	Percent
NS1 antigen	16	12	13	10	6	5	3	2
NS1 IgM	6	5	3	2	1	1	4	3
NS1 IgG	5	4	5	4	2	1	6	5
NS1 IgM, IgG	6	5	2	1	2	1	1	1
IgM	1	1	-	-	1	1	1	1
IgM, IgG	7	5	5	4	3	2	-	-
IgG	10	8	10	8	6	5	7	5
Total	51		38		21		22	

Table 4 Age & gender distribution of serology pattern in relation to population.

Serology pattern	Adult males		Adult females		Pediatric males		Pediatric females	
	Number	Percent	Number	Percent	Number	Percent	Number	percent
NS1 antigen	16/51	31	13/38	34	06/21	29	03/22	14
NS1 with antibody	17/51	33	10/38	26	05/21	24	11/22	50
Antibody	18/51	35	15/38	39	10/21	48	08/22	36

of severe disease in children than adults^[18]. There was no significant difference between the genders.

The immune response model in dengue indicates that NS1 antigen is the earliest marker detected in blood in dengue, between 1-9 days after fever. It is a marker of early diagnosis (acute phase) of disease. IgM antibody is detected from 5th day onwards till 2nd-3rd month, it indicates active/recent infection. IgG antibody from 7th day onwards, decreasing after 3rd month^[1,7,18].

Hematocrit rises due to hemoconcentration attributed to plasma leakage as a result of increased capillary permeability occurring in the critical period. It aids in prognostication and management in dengue^[19,20]. Analysis of our study showed that of 72 cases with a rise in haematocrit, there was a higher proportion in NS1 with antibody (38%) as against the lower proportion (28%) in NS1 antigen only pattern.

IgM is the earliest antibody to be detected from 5th day onwards. The results of our study suggests that the haematocrit rise is established only as a later event in dengue as few studies claimed^[21,22] and is preceded by drop in blood counts^[22]. We could not get data from similar studies to compare our results.

Total leucocyte count - Leucopenia is a major finding in dengue & caused by bone marrow suppression by virus^[4,16]. Leucopenia aids in diagnosis, differentiation & prognostication of dengue^[23,24,25]. In our study, we had 47/132 (36%) of leucopenia cases, 47% of these observed in NS1 antigen pattern whereas 13% were noted in antibody only pattern. There was a strong association with NS1 positivity (NS1 antigen & NS1 antibody) which was in concordance with other studies^[26,27].

In contrast, normal counts were noted more (48%) in the antibody pattern rather than NS1 antigen pattern (22%). Our study indicated that leucocytosis was a later event mostly associated with antibody & none with NS1 antigen, in contrast to few studies which claimed it was an early event^[13].

The results of our study suggests that leucopenia is an early marker of dengue infection as claimed by few studies^[22,26] in view of NS1 antigen association.

Differential count - Bone marrow suppression causes a decrease in polymorphs with increase in lymphocytes especially atypical lymphocytes due to stimulation by nonspecific or specific viral

Table 5 Serology pattern in case with haematocrit rise.

Serology pattern	Number positive	Percentage	NS1 & antibody association
NS1 antigen	20	28	66%
NS1 with antibody	27	38	
Antibody only	25	34	72%
Total	72	100	

Table 6 Normal and increase total counts in association with serology.

Serology pattern	Normal total count		Increased total count	
	Number	Percent	Number	Percent
NS1 antigen	16	22	-	-
NS1 with antibody	22	30	2	17
Antibody only	35	48	10	83
TOTAL	73	100	12	100

Table 7 Serology patterns in decreased total counts (leucopenia).

Serology pattern	Number	Percent	NS1 Ab association
NS1 antigen	22	47	87%
NS1 with antibody	19	40	
Antibody only	6	13	13%
TOTAL	47	100	

Table 8 Serology patterns in association with differential count.

Serology patterns	Neutrophilia		Lymphocytosis*		Association with antibody
	Number	Percent	Number	Percent	
NS1 antigen	2	25	26	30	70%
NS1 with antibody	2	25	27	30	
Antibody only	4	50	35	40	
TOTAL	8	100	88	100	

*lymphocytosis- age adjusted values were used

antigens^[24,27]. Lymphocytosis is a major finding in dengue especially atypical plasmacytoid lymphocytes^[4,11]. The differential count (especially lymphocytosis) helps in differential diagnosis & prognostication in dengue^[4,13,16].

We observed lymphocytosis in 67% of cases. A significant number of these (40%) were associated with antibody only pattern as against

a lower proportion (30%) with NS1 antigen pattern. Neutrophilia was noted in 6% of cases & half the cases were associated with antibody only pattern.

Our study supports the observation of few studies that early in illness there is decrease in neutrophils with lymphocytosis^[27]. Lymphocytosis is a later event predominantly and is established as disease progresses^[13].

We could not get data from similar studies to confirm our findings.

Thrombocytopenia in dengue is caused by bone marrow suppression & immune mediated clearance of platelets^[28]. Thrombocytopenia aids in diagnosis of dengue & helps in its differential diagnosis^[4]. It is useful as a diagnostic, predictive & recovery parameters of DHF^[10].

We had a total of 132 cases of thrombocytopenia. The results in severe thrombocytopenia category showed higher proportion of cases with the antibody only pattern (43%) as against the lower proportion in NS1 antigen pattern (21%). This was in concordance with few studies which claimed increased association of thrombocytopenia with antibody patterns^[8,29]. A few studies noted increased association with NS1 antigen^[1,9]. Others claimed more association of NS1 with antibody^[11]. A few studies have found no correlation between the two^[2]. However, on comparison between studies, it was found inaccurate due to varying thresholds of platelet count, varying methods of correlation, inclusion of normal & high platelet counts in the studies & number of cases included in the study.

The results in our study was in agreement with the observation made in few studies that platelet counts start decreasing by 3rd/4th day to 7th day^{10,30} in view of stronger association with antibody pattern.

Limitations of the study

Our study was limited by: (1) relatively smaller study size; (2) very few similar studies to compare our data & derive conclusions; (3) blood tests were done on random samples & this aspect suffered from lack of standardisation.

CONCLUSION

In India, frequent dengue epidemics strain the limited resources of the public health system. This study explores the utility of simple, easy, rapid & cost effective tests like serology & hematology, also as a supplement & substitute to one other in diagnosis & prognosis of the dengue spectrum especially in rural & peripheral areas.

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