Evaluation of Thrombocytopenia in Dengue Infection in Correlation with Clinical Profile and Immunological Status along with its Seasonal Variation

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ABSTRACT

Background and Aim: Dengue is a rapidly spreading mosquito born Arboviral infection, clinical spectrum of which ranges from self limited Dengue fever (DF) to life threatening severe Dengue, Dengue haemorrhagic fever (DHF) and Dengue shock syndrome(DSS). The aim of study was to evaluate thrombocytopenia and to correlate with clinical profile and immunological status in dengue infection and also to see the seasonal variation of the disease.

Materials and Methods: The present study is a prospective cross sectional study conducted over a period of 2 years, January 2017 to December 2018, in patients admitted in Chalmeda Anand Rao Institute of Medical Sciences, Karimnagar. A total of 1386 suspected fever cases have been studied, out of which 250 were confirmed to be serologically positive as Dengue infection. Platelet count was evaluated by automated cell counter and also confirmed by manual examination by peripheral smear.

Results: A majority of patients were below 30 years with a male preponderance. There was a significant positive predictive value between the age groups and severity of thrombocytopenia. NS1 positivity is seen in 18% of suspected patients, out of which 42% IgM positive and 19.2% IgG positive. Severe thrombocytopenia was seen in patients who were both IgM and IgG positive, with a significant p-value. Also the majority of cases were observed in postmonsoon period with a significant seasonal variation of the disease.

Conclusion: The present study could comprehensively assess the prevalence and severity of thrombocytopenia and its association with clinical and immunological status in Dengue patients along with seasonal variation of the disease.

Keywords: Thrombocytopenia, dengue infection, immunological status, seasonal variation.

INTRODUCTION

Dengue is a self-limited, systemic viral infection transmitted between humans by mosquitoes with a rapidly expanding global footprint.[1] The global burden of dengue is large; an estimated 50 million infections per year occur across approximately 100 countries, with potential for further spread.[2] In India too, dengue is endemic in almost all states and is the leading cause of hospitalization.

Dengue viruses (DENVs) are the most important human Arboviruses worldwide and are transmitted by mosquitoes of the genus Aedes in the form of four distinct serotypes (DENV-1, DENV-2, DENV-3, and DENV-4).[3] Infectious virus and the virus-encoded NS1 are present in blood during the acute phase, and high-level early viremia and NS1 antigenemia have been associated with more severe clinical presentations.[4] The detection of NS1 is also the basis for commercial diagnostic assays.

Thrombocytopenia due to activation of complex immune mechanisms and direct dengue virus action on bone marrow is one of the hallmark feature of dengue.[3] In severe dengue, disseminated intravascular coagulation can contribute to thrombocytopenia.[3]

Platelet count is considered to correlate with the severity of dengue.[2] Serial platelet counts are a key laboratory investigation parameter in managing dengue patients. In some severe dengue cases, a combination of thrombocytopenia with other factors leads to life-threatening hemorrhages[2], hence a major concern to clinicians.

Dengue infection is observed to be a seasonal disease with explosive outbreaks, mostly in urban areas strongly influenced by rainfall and temperature.[5] Also, the El Nino phenomenon may also be related to vector borne diseases transmitted by mosquitoes, such as malaria and dengue.

Despite the increasing magnitude of burden of dengue infection in India, there are relatively few studies in India evaluating thrombocytopenia in dengue infection.[5] Thus the present study aimed to evaluate the prevalence of thrombocytopenia in dengue infection along with its seasonal variation in patients admitted to a tertiary hospital.

Aim and Objectives:

1. Evaluation of thrombocytopenia in patients with dengue infection
2. To study the demographic and clinical profile of patients of dengue infection.
3. To study the seasonal variation of dengue infection.
4. To study its correlation with immunological status
5. To compare with other studies.

MATERIALS AND METHODS

Blood samples were collected from patients with acute febrile illness. Serological confirmation was done using "Rapid Visual test kit" for detection of NS1 antigen and differential detection of IgM and IgG. Platelet count was done using "Automated cell counter XN1000" which was correlated with manual platelet count.

Study Design

The present study was a prospective cross sectional study conducted in Chalmeda Anand Rao Institute of Medical Sciences over a period of 2 years January 2017 to December 2018.

Inclusion criteria

All patients presenting with febrile illness associated with thrombocytopenia and serologically tested positive for dengue infection wil be included .

Exclusion criteria:

Patients with thrombocytopenia but serologically negative, Patients with thrombocytopenia without fever.

Ethical approval :

The study was approved by the Institute Ethics Committee, CAIMS, Karimnagar.

STATISTICAL ANALYSIS

All the Data were entered in MS Excel and analysed by using SSPS Inc, Chicago, IL, USA, version 16. Means, Standard Deviations and Chi- square test were done as applicable. A P value of <0.05 was considered significant and <0. 001 as highly significant.

RESULTS

A total of 1386 patients tested, out of which 250 patients were diagnosed to have Dengue. Minimum age observed was 7 years and maximum age observed was 74 years. Males comprised 52.83% in <18 years age group; there were 59(71.08%) males in 18-30 years age group. Females comprised 25(47.17%) in <18 years age group; there were 24(28.92%) females in 18-30 years age group. (Table1).

Table 1 : Age group wise gender distribution of patients

Age group (Years)	Males	Females	Total
	N (%)	N (%)	N (%)
<18 years	28(52.83)	25(47.17)	53(100)
18-30 years	59(71.08)	24(28.92)	83(100)
31-40 years	33(80.49)	8(19.51)	41(100)
41-50 years	25(64.10)	14(35.90)	39(100)
51-60 years	15(65.22)	8(34.78)	23(100)
>60 years	7(63.64)	4(36.36)	11(100)
Total	167(66.80)	83(33.20)	250(100)

Fever was the most common symptom present in 245(98%) patients followed by severe body ache in 62(24.8%) patients. Bleeding manifestations were present in 23(9.2%) patients and headache was present in 57(22.8%) patients (Table 2).

Table 2: Clinical Features in Patients of Dengue

Clinical Features	N	%
Fever	245	98
Bleeding manifestations	23	9.2
Severe body ache	62	24.8
Headache	57	22.8

Of 250 (100%) patients with dengue; 160 (64%) had thrombocytopenia (platelet count <1 lakh/µL and in remaining 90(36%) patients did not have thrombocytopenia (platelet count >1 lakh/µL).
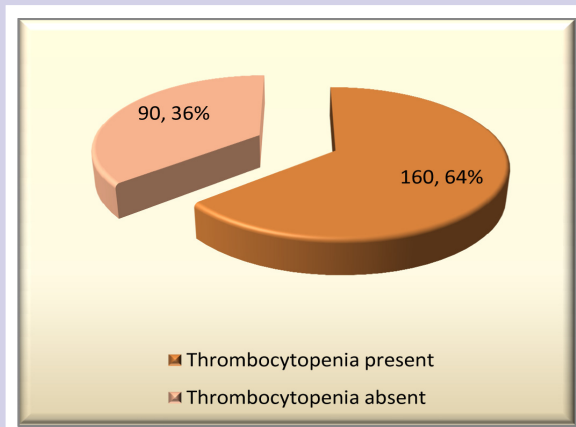

Figure 1: Prevalence of thrombocytopenia in patients with dengue

Of a total of 1386 suspected patients of Dengue; Nonstructural protein 1 (NS1) antigen test was positive in 250 patients and in 1136 patients it was negative. (Figure 2).
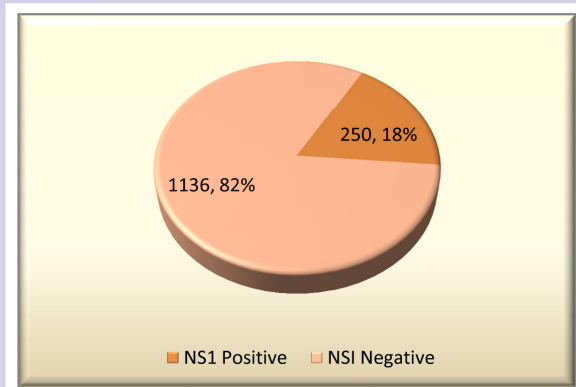

Figure 2: NS1 Positivity in suspected patients of Dengue.

Table 3: Month-wise variation in no. of suspected and confirmed cases of dengue

	Suspected	Confirmed cases		Total
		<18 years	18 years & above	
January	69	6	7	13
February	82	1	8	9
March	86	2	6	8
April	84	3	11	14
May	79	2	14	16
June	98	5	7	12
July	88	2	7	9
August	91	4	9	13
September	126	5	19	24
October	191	13	32	45
November	198	7	35	42
December	194	3	42	45
Total	1386	53	197	250

In the present study, maximum cases were confirmed in October and December and least number of cases were confirmed to be serologically positive in March. Majority of cases, 145 (58%) were confirmed to be serologically positive in the post monsoon period (October - January) (Table 3).

In the present study; the study group (N=250) comprised of 167 (66.8%) males and 83 (33.2%) females. Table 5 shows comparision with other studies.

In the present study and other studies; male preponderance is observed. These observations indicate the gender specific difference among the dengue incidence, which might be related to exposure of dengue vector, might have been contributed by social and cultural reasons.

In the present study, fever was the most common

Table 4: Association of severity of thrombocytopenia with age groups

Age group (yrs)	<20000/µL	20-50000/µL	50000-1 lakh/µL	>1lakh/µL	Total	P value
	N (%)	N (%)	N (%)	N (%)	N (%)	
<18	20(37.74)	12(22.64)	10(18.87)	12(22.64)	53(100)	<0.05*
18-30	2(2.41)	13(15.66)	31(37.35)	37(44.58)	83(100)	
31-40	0(0)	10(24.39)	14(34.15)	17(41.46)	41(100)	
41-50	2(5.13)	9(23.08)	14(35.90)	13(33.33)	39(100)	
51-60	1(4.35)	8(34.78)	6(26.09)	8(34.78)	23(100)	
>60	1(9.09)	4(36.36)	3(27.27)	3(27.27)	11(100)	
Total	26(10.4)	56(22.4)	78(31.2)	90(36)	250(100)	

symptom present in 245(98%) patients followed by severe body ache 62(24.8%), head ache (22.8%), bleeding manifestations (9.2%). Most patients presented with combination of symptoms. Similar findings are reported by other studies: In the study by Raza FA et al. [12] Myalgia was present in 87.3% patients, followed by headache (76.9%) and haemorrhage (47.8%).

In the present study, in out of a total 1386 suspected cases of dengue infection, in 250 (18.03%) patients dengue infection was serologically confirmed by detection of NS1 antigen. Varied rates of seropositivity are reported by other similar studies (Table 6).

Table: 5 Male-female percentage in various studies

Study	Males%	Females%
Khan DM et al[5]	62.6	37.4
Khan E et al[8]	63.2	36.8
Dutta P et al[9]	57.3	42.7
Neralwar et al[10]	63.19	36.8
Desai S et al[11]	58.52	41.47
Raza FA et al[12]	72.9	27.1
Present study	66.8	33.2

Table 6: Percentage of seropositivity in various studies

Study	Percentage (%)
Khan DM et al[5]	7.3
Pruthvi D et al[13]	18.97
Dutta P et al[9]	33.3
Neralwar et al[10]	32.86
Desai S et al[11]	32.72
Present study	18.03

Table 7: Association between IgM and IgG positivity and severe thrombocytopenia

	Serologically confirmed dengue cases	Platelet Count <20000		P value
	N	N	%	
IgM Negative, IgG Negative	136	6	4.41	<0.001**
IgM Positive, IgG Negative	66	4	6.06	
IgM Negative, IgG Positive	9	2	22.22	
IgM Positive, IgG Positive	39	14	35.90	
Total	250	26	100	

Table: 8 Seasonal variations in different studies

Study	No. of cases (Pre-monsoon)	No. of cases (Mon-soon)	No. of cases (Post-monsoon)	Total seropositive cases
Khan DM[5]	15	31	61	107
Pruthvi D[13]	28	107	159	294
Amin MMM[15]	2	3	13	18
Present study	47(18.8%)	58(23.2%)	145(58%)	250(100%)

DISCUSSION

Dengue fever (DF) is one of the most prevalent and fastest spreading mosquito-borne Arboviral infection occurring in tropical and sub-tropical regions around the world and leads to explosive outbreaks in urban areas influenced strongly by rainfall and temperature. It is estimated that more than 50 million infections occur each year, including 500,000 hospitalizations for dengue haemorrhagic fever, mainly among children, with the case fatality rate exceeding 5% in some areas. [2]

DF is a severe flu-like infection that involves individuals of all age groups (infants, children, adolescents, and adults). The dengue virus, a member of the genus Flavivirus of the family Flaviviridae, is an arthropod-borne virus that includes four different serotypes (DEN-1, DEN-2, DEN-3, and DEN-4). Transmission among human beings occurs by the mosquito Aedes aegypti and chiefly occurs during the rainy season.

DENV non structural 1(NS1) antigen is a biomarker for early diagnosis of DENV virus infection. Titres of NS1 represent viral load and viral load is directly proportional to complications. IgM and IgG antibodies are detectable only around 5th day of primary infection and 3rd day of secondary infection. So there is a window period in which IgM or IgG can not be tested in both primary and secondary infection. NS1 is positive from day 1 itself in both primary and secondary infection. IgG is less reliable than IgM. So dengue specific IgM is a good indicator of recent infection and raising titres are important indicators rather than single testing.[6]

In the present study, the mean age of patients was 31.60 (+-14.70) years. Minimum age observed was 7 years and maximum age observed was 74 years. More than half 136 (53.4%) of the patients were </=30 years. Adults comprised of 79.8% of total cases and 53(21.2%) were paediatric cases <18 years.

In the study by Khan DM et al. [5] mean age of the seropositive cases was 29.78 ± 15.3 years and 41(38.91%) patients belonged to the age group of 15-30 years.

In the study by Arya SC et al[7], the patients were aged between 2 and 92 years, mean age 31.2 years with standard deviation 15.5 years.

Thus, in the present study and other studies, the most common affected age group was adults between 20-30 years. In some studies; pediatric population was affected also significantly affected. Affected age group suggests more exposure to vectors, due to increased mobility due to migration and other social factors.

Thrombocytopenia due to activation of complex immune mechanisms and direct dengue virus action on bone marrow is one of the hallmark features of dengue. [3] Platelet count is considered to correlate with the severity of dengue.[2] Serial platelet counts are a key laboratory investigation parameter in managing dengue patients.

In the age group of <18 years there were more patients 20(37.44%) of severe thrombocytopenia as compared to the other age groups. The severity of thrombocytopenia and different age groups were found to be significantly associated. (P<0.05) (Table:4) Simliar results were observed in study by Khan DM et al5 in which also statistically significant association (p<0.05) was found between age groups and severity of thrombocytopenia.

Differences in reported seropositivity rates can be explained by differing periods of studies, geographical variations, outbreaks,[7] differences in inclusion and exclusion criteria.

In the present study; of the 250 patients positive for dengue NS1 antigen; 105(42%) patients were positive for IgM antibodies and 145(58%) patients were IgM negative. Among a total of 105(100%) patients positive for IgM antibodies; 39 (37.14%) patients were positive for IgG antibodies and 66(62.86%) patients were negative for IgG antibodies. Among a total of 145(100%) patients negative for IgM antibodies, 9(6.21%) patients were positive for IgG antibodies and 136(93.79%) patients were negative for IgG antibodies.

Severe thrombocytopenia (platelet count <20,000) was seen in 6(4.41%) patients who were both Ig M Negative & IgG Negative; in 4(6.06%) patients who were Ig M Positive & Ig G Negative; in 2(22.22%) patients IgM Negative & IgG Positive and in 14(35.90%) patients who were both IgM Positive and IgG Positive. IgM and IgG Positivity were found to be significantly associated with severe thrombocytopenia. (P<0.001) (Table 7).

In the study by Kulkarni RD et al[6], association of thrombocytopenia with NS1 was found to be higher (P value<0.001) and excellent when tested with both NS1 and Ig M (P value <0.001). This is in accordance with the present study.

Though dengue virus induced bone marrow suppression decreased platelet synthesis, an immune mechanism of thrombocytopenia caused by increased platelet destruction appears to be operative in patients with DHF. Mourao MP [14] observed that patients with DHF had lower platelet counts than patients with only DF. Moreover, symptomatic thrombocytopenia would require platelet transfusion though platelet counts might not correlate well with clinical bleeding. [15]

In the present study majority of cases confirmed to be serologically positive for NS1antigen in post monsoon period (October-January), 145(58%). Table 8 shows seasonal variation in different studies.

The present study and other similar studies have also provided the evidence for influence of environmental conditions on dengue virus infection. Worldwide studies have proposed that ecological and climatic factors influence the seasonal prevalence of both A.aegypti and dengue virus.

Dengue surveillance and control can be enhanced by wider use of laboratory testing for thrombocytopenia and specific tests for NS1 antigen, IgM and IgG antibodies to confirm dengue, especially during the local dengue transmission season.

CONCLUSION

The present study comprehensively assessed prevalence and severity of thrombocytopenia and its association with immunological status in patients with Dengue infection. The study was conducted over a period of 2 years and the month-wise and seasonal variation could be assessed.

CONFLICT OF INTEREST:

The authors declared no conflict of interest.

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