Original Research Article

Evaluation of thrombocytopenia in dengue infection along with seasonal variation

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Abstract

Introduction: Dengue fever is one of the most prevalent and fastest spreading mosquito borne arboviral infection, occurring in tropical and subtropical regions. They are single stranded RNA viruses and transmitted by Aedes mosquitoes. There are four serotypes of dengue virus DENV 1, DENV2, DENV3, DENV 4. Following an infection lifelong immunity develops against the respective serotype. The clinical spectrum of dengue infection varies from undifferentiated fever, Classical Dengue fever, Dengue hemorrhagic fever, Dengue shock syndrome, Expanded dengue syndrome. Continuous surveillance of dengue fever is important for the proper and timely institution of vector control measures.

Aim: The aim of present study was to evaluate the thrombocytopenia with prevalence of dengue infection along with seasonal variation.

Materials and methods: The present study was a retrospective study conducted in the Department of Hematology, at Chalmeda Anand Rao Hospital, Karimnagar during the period from 2015 June to 2016 May. Blood samples were collected from 4047 patients presented with acute febrile illness clinically consistent with Dengue infection. Serological confirmation of dengue infection was done using "Rapid Visual test kit" for detection of NS1 antigen and differential detection of IgM and IgG. Platelet count was done on "Automatic cell counter XN1000" which was correlated with manual platelet count in all the serologically positive cases.

Results: Out of 4047 suspected cases, 1505 cases were confirmed as serologically positive for dengue infection. Out of 1505cases, 742 samples were positive for only NS1, 70 were positive for only IgM, 361 were positive for only IgG, remaining 332 were positive for more than one serological markers (NS1, IgM, IgG). A majority, 655 (43.52%) of the dengue cases were noted in the age group of 15-30 years, followed by less than 15 years of age. Among the dengue cases, 61.8% were males and 38.2%

were females. A significantly higher number of serologically positive cases 1342 (89.2%) were noted in the post monsoon period.

Conclusion: Incidence of dengue infection was higher in monsoon and post monsoon period. Thrombocytopenia provide high suspicion of dengue infection, which could be life threatening. Platelet count is an important predictive and recovery parameter of dengue infection.

Key words

Dengue fever, NS1 antigen, IgM and IgG antibody, Seasonal, Platelet count.

Introduction

Dengue virus is a single-stranded RNA viruses that belong to the family Flaviviridae and the genus Flavivirus. It is an emerging viral infection causing potential danger globally. It can cause simple fever to life threatening complications. The complications such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) are usually related to cross-reactivity between one serotype to other [1]. Distribution of dengue infection is associated with the day time biting mosquito Aedes aegypti. Dengue Infection is observed to be a seasonal disease. In tropical areas, as the vector is active throughout the year so as the disease but with maximum transmission and disease incidence in months of rainfall [2]. Rainy season and post rainy season favors the collection of water in various sites like old tires, coolers, old earthenware pots, etc., which acts as a good site for mosquito breeding [3]. During the rain fall the mosquito population is high (3-4 female mosquitoes per house) as compared to the density during the dry season (1-2 female mosquitoes per house) [4].

Identification of Dengue Specific IgM and IgG antibodies in combination with detection of NS1 antigen in serum samples is the mainstay of diagnosis. Secretory protein NS1 antigen is seen in high concentrations during acute phase of illness 1 to 5 days [5], but Dengue Specific IgM and IgG antibodies appear only after 4 to 6 days of illness [6]. Isolation of virus, RT-PCR is time consuming and requires costly laboratory methods, so large number of cases identified by using a cost effective Rapid Visual test kit for IgM, IgG and NS1antigen specific to virus,

which remains as important diagnostic parameters for early detection of disease [7].

During first 3 days of illness platelet count is normal, thrombocytopenia begins during febrile phase and platelet count is progressively reduced during hemorrhagic illness (DHF) [8]. As per WHO guidelines, thrombocytopenia can be used as simple diagnostic criteria for DHF which is roughly estimated by microscopy even in the peripheral laboratories [9, 10]. Spontaneous bleeding is noted in platelet count of <20,000 in majority of patients and petichae/ purpura is seen in platelet count in the range of 20,000-40,000,which signifies the need to evaluate platelet count and follow up after platelet transfusion thereby curtailing further progression of disease to its severe forms and thus increasing positive prognosis.

Materials and methods

The present study was a retrospective study conducted in the Department of Hematology, at Chalmeda Anand Rao Hospital, Karimnagar during the period from June 2015 to May 2016. Blood samples were collected from 4047 patients presented with acute febrile illness clinically consistent with Dengue infection. Serological confirmation of dengue infection was done using immune-crhomatographic card test for detection of NS1 antigen and differential detection of IgM and IgG antibodies. Platelet count was done on "Automatic cell counter XN1000" which was correlated with manual platelet count in all the serologically positive cases.

Inclusion criteria

All patients with clinical features and serologically positive dengue infections were included.

Exclusion criteria

- Patients with thrombocytopenia but serologically negative for dengue infection were not included.
- Patients with thrombocytopenia and no fever were not included.

Results

Out of 4047 suspected cases, 1505 cases were confirmed as serologically positive for dengue infection. Out of 1505 cases, 742 samples were positive for only NS1antigen, 70 were positive for only IgM antibody, 361 were positive for only IgG antibody, remaining 332 were positive for more than one serological markers (NS1, IgM, IgG) as shown in **Table - 1**. A majority, 655 (43.52%) of the dengue cases were noted in the age group of 15-30 years, followed by less than 15 years of age 440 cases(43.52%). Among the dengue cases, 925 (61.46%) were males and 580 (38.53%) were females as shown in **Table - 2**.

Table-1:Seropositivity of different parameters for Dengue infection	
Serological markers	**Number of positive**
NS1	742
IgM	70
IgG	361
NS1+IgM	50
NS1+IgG	202
IgM+IgG	50
NS1+IgM+IgG	30
Total	1505

Table-2:Relation of seropositivity of Dengue infection with Age & Sex			
Age group	Male	Female	Total
<15yrs	280	160	440
15-30yrs	405	250	655
31-50yrs	180	130	310
>50yrs	60	40	100
Total	925	580	1505

Table-3:Platelet count comparison in seropositive cases			
<25,000 cells/cumm	25,000-50,000 cells/cumm	50,000-1,00,000cells/cu mm	1,00,000-1,50,000cells/cu mm
298	644	406	157

Table-4:Seasonal distribution of dengue seropositive cases		
Month	Total tested	Positive
June, 2015	230	50
July	350	200
August	647	282
September	650	300
October	550	255
November	450	200
December	400	105
January, 2016	370	50
February	130	35
March	120	10
April	80	08
May	70	10
Total	4047	1505

According to the present study during the monsoon period that is from June to September 832 cases (55.28%), post monsoon period from October to January 610 cases (40.53%) and pre-monsoon period from February to May 63 cases (4.18%) were confirmed serologically positive as shown in **Table - 4**.The difference between numbers of serologically positive cases in the monsoon and post-monsoon period was significantly higher as compared to serologically negative cases.

Platelet count was done in all the serologically positive cases out of which 298 cases (19.80%) the platelet count was <25,000. 644 cases (42.79%) ranged from 25,000-50,000. 406 cases (26.97%) ranged from 50,000-1,00,000. 157 cases (10.43%) ranged from 1,00,000-1,50,000 as shown in **Table - 3**.

Discussion

A total of 1505 seropositive cases were noted among 4047 patients experiencing a febrile illness, clinically consistent with Dengue infection admitted during the study period from 2015 June to 2016 May. In the present study, many patients with serological confirmation were in the age group of 15-30 year, followed by less than 15 years of age and this is in accordance with the study done by Khan, et al. [11]. A majority, 655 (43.52%) of the dengue cases were noted in the age group of 15-30 years, followed by less than 15 years of age 440 cases (43.52%). Study by Khan, et al. shows 41 (38.3%) dengue cases in the age group of 15 – 30 years, followed by less than 15 years of age 29 cases (27.1%). The mean age of the seropositive cases was 29.78 ± 15.3 years.

Out of 1505 seropositive cases, 925 (61.46%) were males and 580 (38.53%) were females, the male to female ratio was found to be 1.6 : 1 and is consistent with the study conducted by Asmabegaum, et al. [12] and Khan, et al. [11]. In the present study, most patients with serological and virological confirmation were in the age group of 15-30 years and this is in accordance with the study by Khan et al. [11].

Majority of the patients were found to be NS1 antigen positive in acute phase and this is in accordance with the study done by Trupti [13] and Asmabegaum, et al. [12]. Out of 1505cases, 742 samples were positive for only NS1, 70 were positive for only IgM, 361 were positive for only IgG, remaining 332 were positive for more than one serological markers (NS1, IgM, IgG). During first few days of illness NS1 Ag circulates at

high level in our blood. The level of NS1 Ag varies from 0.04 - 2 μg/ml in acute phase serum samples and only 0 .04μg/ml or even less in convalescent phase. Hence, NS1 antigen positivity in a person indicates acute phase of illness [14]. In our study 49.30% of cases were positive only for NS1 antigen. Complications associated with dengue fever can be avoided by using NS1 antigen assay because it can be identified in serum from day one of fever [15]. Dengue specific IgM can be detected in blood only after 3 to 5 days of illness, hence it cannot be used as an early diagnostic marker. IgG antibody is not a reliable marker as it is a cross reacting antibody to other flavi viruses.

Platelet count was done in all the serologically positive cases i.e.1505 cases, out of which 298 cases (19.80%) the platelet count was <25,000. 644 cases (42.79%) ranged from 25,000-50,000. 406 cases (26.97%) ranged from 50,000-1,00,000. 157 cases (10.43%) ranged from 1,00,000-1,50,000. In our study large proportion of patients with platelet count<50,000cells/cumm were found and is consistent with the study conducted by Carmen, et al. [16].

As far the seasonal variation was concerned most cases were seen during the monsoon period that is from June to September 832 cases (55.28%) and post monsoon period from October to January 610 cases (40.53%). The difference between numbers of serologically positive cases in the monsoon and post-monsoon period was significantly higher as compared to serologically negative cases .The rise in incidence of dengue infection in monsoon and post monsoon period, which is supported by a study done by Carmen, et al. In most countries dengue epidemics are reported to occur during the warm, humid, and rainy seasons, which favour abundant mosquito growth and shorten the extrinsic incubation period as well [17-19]. Transmission of dengue increases during monsoon and the post monsoon period of subnormal rainfall. The temperature remains high during the pre-monsoon period. It is continuous rain pour for a couple of days that brings down the temperature during the monsoon period, which may also be responsible for an increase in the relative humidity and decrease in the evaporation rate thus maintaining secondary reservoirs containing rain water. Comparative study of authors of various parameters was as per **Table – 5**.

Table - 5: Comparative study of authors of various parameters.

Names	Age group	Sex	Platelet count	Months	Serological Marker
Pruthvi, et al. [9]	<12 yrs	------	< 25,000	Aug - Oct	IgM
Asmabegaum, et al. [12]	0-15 yrs	M > F	< 1 lakhs	Aug - Oct	NS1
Carmen, et al. [16]	20-29 yrs	M > F	< 50,000	July - Dec	NS1
Khan, et al. [11]	15-30 yrs	M > F	51,000 – 1 lakh	Sep - Nov	NS1
Trupti Dongre, et al. [13]	----	M > F	> 75,000	Sep- Nov	NS1
Present study	15-30yrs	M > F	< 50,000	July - Dec	NS1

Conclusion

There is a strong relation between thrombocytopenia with dengue fever which could be life threatening. Platelet count is an important predictive and recovery parameter of dengue infection. The present study highlighted significant dengue infection during seasonal variation. NS1 antigen identification is best parameter for diagnosis of infection in initial phase of illness as it will be positive from day 1 of dengue infection. Early recognition and prompt management is essential to reduce the morbidity and mortality associated with dengue infection.

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