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Virulence of Dengue Fever and Bleeding Tendency: Understanding Clinical and Laboratory Manifestations

Authors
Dr Praveen Kumar Garg[1], Dr Ambadan Rao[2]
[1]Assistant Professor, Department of Medicine, Government Medical College, Pali (Rajasthan)
Email: *gargdrpraveen@gamil.com*
[2]Radiologist (MD), Government Bangur Hospital Pali, (Raj.)
Corresponding Author
Dr Ambadan Rao
Radiologist (MD), Government Bangur Hospital Pali, (Raj.)

Abstract
Background: *Dengue is known to have multifarious health and economic impacts, due to lack of specific therapy for dengue virus infection and outcome is dependent on medical care received. Therefore, the present study will attempt to establish an association of thrombocytopenia due dengue infection and MPV diagnosis.*
Material & Methods: *All those patients who are going to get admitted as in patients in Government Bangur Hospital, Pali (raj.) with symptoms suggestive of Dengue fever such as fever, severe headache, joints pain and bleeding manifestations, shock are investigated with Dengue rapid test. A detailed clinical history is taken from all patients followed by thorough clinical examination of all systems.*
Results: *In present study showed that 205 dengue positive patients. Out of 205, 70 DHF patients, 80 DF and 55 DSS (and bleeding) patients. The majority of cases were female (51.25% & 52.72% respectively) in DHF & DF, but mostly male patients (55.75%) occurred with DSS. The mean age of patients was third decade of years (range 22-69 years). Mean value of platelet count in DF, DSS & DHF was 80.0 ± 4.9, 36.0 ± 6.3 & 39.7 ± 4.9 respectively. But not statistically significant (P=0.21).*
Conclusion: *With the objective of studying mean platelet volume in association with severity, serology & treatment outcome, the study produces equivocal results on MPV's prognostic utility. MPV showed no significant correlation with severity, serology.*
Keywords: *Dengue fever, Dengue Shock syndrome, Dengue hemorrhagic fever, MPV.*

Introduction

Dengue as a condition has been recognized as the most common mosquito-borne viral disease affecting humans worldwide, especially in tropical and subtropical regions and caused by dengue virus which belongs to the Flaviviridae family of positive-sense single-stranded RNA viruses. With 4 serotypes, this virus is known to cause a broad spectrum of clinical manifestations in humans[1]. Infection can be asymptomatic or cause a range of manifestations in severity. From a mild febrile illness called dengue fever to life-threatening complications known as dengue hemorrhagic fever (DHF) which can then progress to dengue shock syndrome (DSS)[2,3] due to accompanying hematological complications. The exact

mechanism of dengue hemorrhagic fever hasn't been well documented though it is thought to involve immunopathological processes associated with sequential infections with different virus serotypes[4]. Viral virulence, genetic and acquired host factors are all known prognosticators that estimate the dengue severity[5,6] whereas more virulent forms of the disease with hematological manifestations are related to an alteration of the Th1/Th2 immune reaction[1].

Epidemiologically speaking, for the last half of the century, the globe has witnessed a 30-fold increase in incidence and geographic expansion to new countries[7]. In 2016 annual estimates of dengue were found to be in millions with approximately 2.5 billion people known to be residents of areas endemic to dengue[8]. 2009 estimates on Dengue showed resurgence of dengue in India in recent times where case fatality rates are 3-5 %[9]. In 2015, dengue it was reported, caused about 100 million asymptomatic cases and 25 thousand deaths[10]. Dengue is known to have multifarious health and economic impacts, due to lack of specific therapy for dengue virus infection and outcome is dependent on medical care received. This can also be attributed to the uncertainty about protective versus pathogenic immunity in dengue infections[11].

Thrombocytopenia is the most common clinical identification of the dengue virus infection and it leads to bleeding although the platelet count may not directly correlate with the bleeding manifestation. Platelet indices such as platelet volume (a marker of platelet function and activity), is measured as mean platelet volume (MPV) and is an independent predictor of bleeding; are now being investigated as prospective platelet activation markers[8,12]. Though the study of platelets may have a substantial impact on reducing the mortality and morbidity associated with dengue yet it is important to note that MPV as a diagnostic tool in different conditions with thrombocytopenia produces counterproductive results for the sensitivity and specificity varies with the condition it is being associated with. Therefore, the present study will attempt to establish an association of thrombocytopenia due dengue infection and MPV diagnosis. It has also been demonstrated that in hospital based setting, number of dengue patients presented with bleeding tendency in the absence of vascular leakage or hemoconcentration[13,14]. Therefore, even with diagnosis now being driven by WHO definitions of the condition, there has risen a need for standardizing DHF definition to clarify the disease severity of dengue fever. Multiple definitions are used to define DHF and DSS in various clinical studies and they are known to exhibit different clinical and laboratory manifestations with regard to bleeding time, liver function, frequency of secondary viral infection and Th1/Th2 cytokine profiles[1]. The present study will tend to clarify the distinction between clinical and laboratory data between DF, DHF and DSS to provide a better insight into the pathogenesis of varying dengue severity. This may lead to revised strategies for the prevention and treatment of more virulent forms of the disease. The objectives of this study as follows:

- To evaluate the correlation of bleeding severity with platelet count.
- To evaluate the correlation of platelet volume at the time of bleeding.

Material & Methods

Source of data: All those patients who are going to get admitted as in patients in Government Bangur Hospital, Pali (raj.) with symptoms suggestive of Dengue fever such as fever, severe headache, joints pain and bleeding manifestations, shock are investigated with Dengue rapid test. Those patients found positive for the test are included in study and informed consent is taken from all patients.

A detailed clinical history is taken from all patients followed by thorough clinical examination of all systems. They are further investigated with other biochemical, microbiological, haematological, radiological investigations mentioned in study protocol.

Method of collection of data:

The data for study was collected from subjects fulfilling inclusion criteria/exclusion criteria and admitted in Government Banur Hospital, Pali (Rajasthan).

Sample Size: 205 cases

Duration of study: May 2015 to June 2017

Study Design: A Descriptive study

Sampling Method: Purposive sampling

Inclusion criteria: All the adult patients with clinical features suggestive of Dengue infection, confirmed by Dengue serology (NS1, IgM & IgG) was included in this study.

Exclusion criteria:

1. Mixed infections were excluded from the study
2. Chronic alcoholics were excluded from the study
3. CLD cases
4. ITP, TTP due to any cause
5. Septicemia due to any cause

Serological tests

A. MAC ELISA

The assay is based on entrapping human IgM antibodies on a microtiter plate using anti-human-IgM antibody followed by the addition of dengue virus specific antigen (DENV1-4).

IgG ELISA

The IgG ELISA used for the detection of a past dengue infection utilizes the same viral antigens as the MAC ELISA. This assay correlates with the hemagglutination assay (HI) previously used. In general IgG ELISA lacks specificity within the flavivirus serocomplex groups. Primary versus secondary dengue infection can be determined using a simple algorithm.

NS1 ELISA

The non-structural protein 1 (NS1) of the dengue viral genome has been shown to be useful as a tool for the diagnosis of acute dengue infections. Dengue NS1 antigen has been detected in the serum of DENV infected patients as early as 1 day post onset of symptoms (DPO), and up to 18 DPO.

Measurement of blood for clinical manifestations

As adapted from precious study protocols, heparinized blood samples (5 ml) from patients hospitalized with febrile dengue virus infections were collected on subsequent day of admission and then on the 7th day. Through centrifugation, plasma samples were collected and the stored for analysis and at −80-degree Celsius. Blood cytokines, including IFNγ (pg/ml), IFNα (pg/ml), soluble vascular cell **a**dhesion molecule I and IL-10, were measured **u**sing standardized procedures and kits. Analysis is based on values; summarized by the interpolation of curves as devised by the manufacturers of these standardized procedures and kits[1]**.**

Determination of primary and secondary Infections

She and his colleagues recommended detection of dengue antibodies for reporting diagnostic procedures for differentiation of primary and secondary viral infections[15,16]. The study uses immunoassay to differentiate between primary and secondary infections in blood samples collected on the second and seventh day of the disease. Definition of primary dengue virus infection is detection of virus RNA or detectable dengue IgM, whereas secondary infection is defined by both detectable dengue IgG and RT-PCR detection of dengue virus RNA in the blood within 7 days of the disease. The cut-off value for the detectable capture virus IgG antibodies is usually presented as the antibody index (AI). A previous study set the cut-off value for the AI for a positive detection of capture IgG was set at 22 and has been adopted for current analyses[16].

The study was designed in congruence with Declaration of Helsinki[17], and made and a careful assessment of all predictable risks was accounted as the benefits were evaluated. Participants of the study were well and their informed consent recorded[18].

Statistical Analyses

The clinical features of patients if numerical were presented as mean ± SEM and the categorical

variables were presented as numbers (percent). The categorical features were compared among groups (DF, DSS and DHF) using Chi square test. Results from the measurement of numerical characteristics of the patients were also documented among patients with DF, DSS and DHF and then analyzed by ANOVA. Student's t-test was used to analyze differences in clinical features among those with primary and secondary infection and between those non-vascular and vascular leakage groups. A two-tailed p-value <0.05 was considered statistically significant. IBM SPSS version 23.0 was used for the analysis.

Results

In present study showed that 205 dengue positive patients. Out of 205, 70 DHF patients, 80 DF and 55 DSS (and bleeding) patients. The majority of cases were female (51.25% & 52.72% respectively) in DHF & DF, but mostly male patients (55.75%) occurred with DSS. The mean age of patients was third decade of years (range 22-69 years) (table 1).

Mean value of platelet count in DF, DSS & DHF was 80.0 ± 4.9, 36.0 ± 6.3 & 39.7 ± 4.9 respectively. But not statistically significant (P=0.21) (table 1).

In present study observed that the higher proportion of patients had mean platelet volume levels from 8 to 12 fL (50.73%), followed by more than 12 fl (45.85%) at the time of admission in hospital and MPV range more than 12fL (55.60%) followed by (44.39%) at time of discharge of patients. However, the mean platelet volume when cross tabulated with severity of dengue fever did not reach statistical significance (P=0.15 & P=0.18 respectively) (table 1).

Our results showed that the mean platelet volume with serology. Out of 205, 191 patients have serology positive & rest were serology negative (14 cases) at the time of admission. Out of 191, 93 patients had mean platelet volume levels from 8 to 12 fL, 91 patients had more than 12 fL and 7 patients had less than 8 fL. IgM antibody were significantly associated with MPV (fL) (P=0.029) but IgG antibody not statistically significant (p=0.13) with MPV (fL) at the time of admission.

Out of 205, 192 patients have serology positive & rest were serology negative (13 cases) at the time of discharge. Out of 192, 111 patients had mean platelet volume levels from more than 12 fL, 81 patients had 8- 12 fL and none of patients had less than 8 fL. IgM & IgG antibodies were statistically significantly associated with MPV (fL) (P=0.05 & P=0.034 respectively) at the time of discharge. Serum positivity and negativity was also put to test in both phases and revealed significant differences at the end of the recovery phase from dengue (table 2).4

Table 1: Demographic and laboratory information of the included patients

Variables	Overall	Dengue Fever (DF) (n=80)	Dengue shock syndrome (DSS) or bleeding (n=55)	Dengue hemorrhagic fever (DHF) (n=70)	p-value
Demographic					
Male	104 (50.73%)	39 (48.75%)	26 (47.27%)	39 (55.71%)	**0.54**
Female	101 (49.26%)	41 (51.25%)	29 (52.72%)	31 (44.28%)	
Mean Age	**34.2 yrs**	**33 yrs**	**35yrs**	**36yrs**	0.25
Platelet count ($\times 10^9$/l)	49.8±5.7	80.0 ± 4.9	36.0 ± 6.3	39.7 ± 4.9	0.021
MPV (fL) (corresponding to minimal platelet counts)					
<8	7 (3.41%)	1 (1.3%)	3 (4.3%)	3 (5.45%)	**0.15**
8 to 12	104 (50.73%)	45 (56.3%)	28 (40%)	31 (56.36%)	
>12	94 (45.85%)	34 (42.5%)	39 (55.7%)	21 (38.18%)	
Total	205	80 (100%)	70 (100%)	55 (100%)	
MPV (fL) at day of discharge					
<8	0 (0%)	0 (0%)	0 (0%)	0 (0%)	**0.18**
8 to 12	91 (44.39%)	36 (45%)	34 (48.57%)	21 (38.18%)	
>12	114 (55.60%)	44 (55%)	36 (51.42%)	34 (61.81%)	
Total	205	80 (100%)	70 (100%)	55 (100%)	

Table 2: Platelet count in patients at the time of admission

Platelet count /L	At the time of admission Number	Percentage
5000-20000	22	10.73%
20000-50000	49	23.90%
50000-100000	97	47.31%
>100000	37	18.04%
Total	205	100%

Table 3: Mean platelet volume with serology

MPV (fL) (corresponding to minimal platelet counts)	Total	IgM		IgG		Sero-Negative	Sero-Positive
		-	+	-	+		
<8	7	0	7	0	7	0	7
8 to 12	104	19	85	24	80	11	93
>12	94	5	89	10	84	3	91
Total	205	24	181	34	171	14	191
Chi-Square		8.97		5.6		4.48	
p value		0.029		0.13		0.21	
MPV (fL) at day of discharge	Total	IgM		IgG		Sero-Negative	Sero-Positive
		-	+	-	+		
<8	0	0	0	0	0	0	0
8 to 12	91	21	70	21	70	10	81
>12	114	8	106	11	103	3	111
Total	205	29	176	32	173	13	192
Chi-Square		12.72		8.63		11.96	
p value		0.05		0.034		0.05	
MPV is corresponding to the lowest platelet counts of the patient, attained during his/her course of illness (before day of discharge)							

Discussion

Dengue as a febrile illness caused by the dengue virus is transmitted to humans by a mosquito vector, and is endemic in over 100 countries[13]. The standardization of diagnosis for dynamic virulence of the condition has been source of concern for one form is usually a self-limiting febrile illness, whereas the other can cause a life-threatening disease[19]. The current study validates the current WHO guidelines and shows the applicability of using multiple diagnostic features for the virulent forms as some manifestations may delay recognition of the potentially fatal condition. Braga et al in Brazil and Harris et al in Nicaragua provided evidence that clinical bleeding presentation in dengue can vary with outbreaks and this warrants for the need for specific classification of dengue severity[20-22]. Thrombocytopenia has been registered as a warning sign and our study shows that other measures like heart rate (tachycardia) >92 beats/means and narrowing blood pressure (< 45 mmHg) are other harrowing signs of the virulence of the condition.

In present study showed that 70 DHF patients long with 80 DF and 55 DSS (and bleeding) patients. Age ranged from 22 years to 69 years. Study population did not vary significantly for gender and age (p=0.54 & p=0.25). Keshava H. K et al (2014)[23] reported 21-30 years of age was most commonly affected, another study done by Varsha Shah, Uresh Jain (2016)[24] found Maximum patients were in 18–30 year's age group (70.86%).

In a resource-limited setting, as suggested in previous studies[10,25], where specific diagnostic tests are seldom available and WHO criteria on focuses entirely on the hematological abnormal-lities in the context of hemoconcentration, with rapidity of decline in platelet numbers; we may

suggest that with thrombocytopenia, other physiological markers can be added to current screening list; there by identifying patients with higher risk of severe disease.

In our study showed that the mean value of platelet count in DF, DSS & DHF was 80.0 ± 4.9, 36.0 ± 6.3 & 39.7 ± 4.9 respectively. But not statistically significant (P=0.21). Our finding suggested the platelet count were less than 50000/L in 71 (34%) patients at the time of admission (table 2). Varsha Shah1, Uresh Jain (2016)[24] found that thrombocytopenia (platelet count <50,000/cumm) at presentation in 68 (45.03%) patients.

Our results showed that the mean platelet volume with serology (table 3). Out of 205, 191 patients have serology positive & rest were serology negative (14 cases) at the time of admission. Out of 191, 93 patients had mean platelet volume levels from 8 to 12 fL, 91 patients had more than 12 fL and 7 patients had less than 8 fL. IgM antibody were significantly associated with MPV (fL) (P=0.029) but IgG antibody not statistically significant (p=0.13) with MPV (fL) at the time of admission.

Out of 205, 192 patients have serology positive & rest were serology negative (13 cases) at the time of discharge. Out of 192, 111 patients had mean platelet volume levels from more than 12 fL, 81 patients had 8- 12 fL and none of patients had less than 8 fL. IgM & IgG antibodies were statistically significantly associated with MPV (fL) (P=0.05 & P=0.034 respectively) at the time of discharge. Afsar et al are of the opinion that high MPV is an indicator of platelet activation and may be used as an initial marker to suspect dengue fever in a case of thrombocytopenia[26]. A significant serological change in immunoglobulins with MPV category has been demonstrated in the present study. A small sample size, validation, limited geographic coverage, absence of repeatability statistics and use of data in cross- sectional study design are major limitations of our study.

Conclusion

With the objective of studying mean platelet volume in association with severity, serology & treatment outcome, the study produces equivocal results on MPV's prognostic utility. MPV showed no significant correlation with severity, serology. Mean platelet volume with thrombocytopenia be an important prognostic parameter in dengue fever. The study provides an insight about the different manifestations of bleeding tendency and vascular leakage with virulence of disease which can be used to increase specificity of the diagnostic criterion when severity of condition needs to be identified.

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