Research Article

IMPORTANCE OF PLATELET COUNT IN DIAGNOSIS OF DENGUE INFECTION

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Abstract- Introduction: Dengue is an acute, potentially fatal viral infection that can culminate into dengue hemorrhagic fever and dengue shock syndrome. It has emerged as a notable public health problem in recent decades in terms of the mortality and morbidity associated with it. Specific antibody Ig M and Ig G detection along with the newer parameter NS1 antigen detection has been the main stay of diagnosis. Apart from these parameters, thrombocytopenia is a constant finding and therefore platelet count can be used as an accessory test for diagnosis of dengue infection in the laboratories where resources are limited. Materials and Methods: A cross-sectional study was conducted using secondary data of clinically suspected dengue cases reported to the microbiology laboratory during June-August months of 2017 at CIMS teaching hospital, Chamarajanagar. Samples were tested either for NS1or IgM by ELISA depending on duration of fever and platelet counts were obtained. Statistical analysis was done using WHO Epi info software version 3.5.4. Results: Out of 1653 clinically suspected cases, 265(16.03%) were found to be positive for dengue infection either by NS1 or IgM ELISA. Among them 135 (50.95%) were females and majority 211 (79.62) were below the age of 30 years. Proportion of thrombocytopenia was significantly higher in dengue positive cases than in negatives. Conclusion: In resource limited setting thrombocytopenia can be used as predictor of dengue infection for early initiation of therapy to reduce the morbidity and mortality associated with it.

Keywords- Dengue, IgM, NS1, Platelet count

Introduction

Dengue is an acute, potentially fatal viral infection that can culminate into dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). It is caused by five serotypes of dengue virus (DV), namely DEN-1, DEN-2, DEN-3, DEN-4 and DEN-5 [1] belonging to genus Flavivirus and family Flaviviridae. It spreads through the bite of infected Aedes aegypti mosquito. Most of the primary infections are uneventful. Infection with one serotype confers an individual life-long immunity to that serotype and cross-reactivity to the other serotypes, which is the basis for the complications like DHF and DSS [2]. Dengue affects up to 100 million people annually, with 5, 00,000 cases of DHF/DSS leading to around 30,000 deaths, mostly among children [3]. It is almost endemic throughout India. Due to rapid urbanization, lifestyle changes and poor water management techniques, the risk of dengue infection in India has increased in recent years [4]. Dengvaxia(R) is a live attenuated tetravalent vaccine for dengue that is currently under evaluation in phase 3 clinical trials in Asia (Indonesia, Malaysia, Philippines, Thailand and Vietnam) and Latin America (Brazil, Colombia, Honduras, Mexico and Puerto Rico). The protective efficacy of Dengvaxia(R) has been estimated to range between 50.2% and 76.6% for different ages and serotypes [5]. It is well known that early and specific diagnosis of DHF or DSS followed by supportive therapy reduces morbidity and mortality [6]. Virus isolation in cell culture, identification of viral genomic sequence by nucleic acid amplification techniques like RT-PCR, NASBA (Nucleic acid based amplification) and detection of dengue specific IgM, IgG antibodies by ELISA (Enzyme linked immunosorbent assay) or by immunochromatographic methods are being used currently in the laboratories for diagnosis of dengue infection [7]. Virus isolation and molecular techniques cannot be used as routine diagnostic tests because they are laborious, time consuming and require specialized laboratory facilities [8]. Serological diagnosis can be done only after appearance of antibodies which usually takes 4-6 days of illness [9]. Combination of NS1 (Non structural protein) antigen detection along with antibody

detection increases the diagnostic rates as secretory protein NS1 antigen is seen in high concentrations during acute phase of illness (1 to 5 days) [10,11]. Immunochromatographic detection of these serological markers yield rapid results but have low sensitivity as compared to ELISA [12]. During febrile period thrombocytopenia starts and during DHF platelet count reduces progressively.[13]. A drop in platelet count below 1,00,000 per mm^3 is usually found between the third and eighth day of illness as per WHO guidelines, so thrombocytopenia can be used to detect and monitor DHF.[14] Platelet count can be roughly estimated by microscopy even in the peripheral laboratories and it is the only accessory laboratory test which supports the diagnosis of dengue [15] Further the resource poor health care system has to depend upon simple to perform and easy to interpret laboratory tests for diagnosis. With this background we tried to correlate the dengue serological markers with platelet counts.

Objectives

To find the seroprevalence of dengue infection in the study setting.

To find out the association between platelet count and serological markers (NS1ag and IgM) in the acute stage of dengue infection.

Materials and Methods

A cross-sectional study was conducted using secondary data of clinically suspected dengue cases who attended various clinical departments of our hospital and reported to the microbiology laboratory during June-August months of 2017 at tertiary care hospital of Chamarajanagar Institute of Medical Sciences, Chamarajanagar. Permission for the study was taken from the head of the Institute. As per WHO guidelines samples of patient < 5 days of fever were tested for NS1Ag by Tulipqualisa and ≥ 5 days of fever for IgM by MAC ELISA, developed by NIV (National Institute of Virology), Pune.

Manufacturer's instructions were followed strictly while performing the ELISA. Platelet counts of the suspected patients were recorded, irrespective of positive or negative result for the above test.

Seroassays

Approximately 5 ml of blood samples were collected from all the suspects of Dengue as a part of the routine laboratory work and the sera were separated and tested for the Dengue NS1 and IgM by ELISA methods.

NS1 Ag assay

NS1Ag tulipqualisa test kit was used to perform the test. NS1Ag tulipqualisa is a solid phase enzyme linked immunosorbent assay (ELISA) based on the "Direct Sandwich" principle. Anti-Dengue NS1 antibodies were coated on microwells with high reactivity for Dengue NS1 antigen. After adding the samples to the wells, enzyme conjugate (monoclonal anti-dengue NS1 antibodies linked to Horseradish peroxidase (HRPO) were added. A sandwich complex was formed in the well wherein dengue NS1 (from serum sample) is "trapped" or "sandwiched" between the antibody and antibody HRPO conjugate. Wash buffer was added that will wash off unbound conjugate. The amount of bound peroxidase was proportional to the concentration of dengue NS1 antigen present in the sample. Blue colour developed on addition of the substrate buffer and chromogen. The intensity of colour was proportional to the concentration of dengue NS1 antigen. To limit the enzyme-substrate reaction stop solution was added, and yellow colour which developed was read at 450 nm spectrophotometrically. Results were interpreted as per the manufacturer's guidelines.

Detection of IgM

Samples of patient ≥ 5 days of fever were tested for the presence of dengue specific IgM antibodies by using MAC ELISA, developed and commercialized by NIV (National Institute of Virology), Pune, and recommended by National Vector Borne Disease control programme. Tests were done and results were read as per the literature provided.

Platelet Count

EDTA blood samples were collected and the platelet count was done by automated analyzer and cross checked by light microscope and interpreted as normal, when the count was between 1,50,000-4,50,000/mm³ and DHF, when the count was < 1,00, 000/mm³ (WHO cut off for platelet count for DHF). Statistical analysis: Statistical analysis was done using WHO Epi info software version 3.5.4.

Results

[Table-1] Diagnosis of dengue infection by dengue specific parameters. Out of total 1653 samples tested, 751 were tested for NS1only and 902 samples were tested for IgM only depending on the stage of illness. Out of 1653 clinically suspected cases, 265 (16.03%) were found to be positive either by NS1 or by IgM ELISA. Rate of seropositivity was 228 (30.35%) for NS1 and for IgM was 37 (4.10%) as evident from the table. [Table-2] and [Fig-1] shows gender wise distribution of Dengue suspects and positives. Out of the total 265 dengue positive cases, 130 (49.05%) were males and 135 (50.95%) were females. Of the 228 positive cases detected by NS1, 111 (48.68%) were males and 117 (51.31%) were females. Similarly, out of the total 37 positive cases diagnosed by IgM, 19 (51.35%) were males and the rest 18 (48.64%) were females. [Table-3] and [Fig-2] shows age wise distribution of Dengue suspects and positives. Of the 265 total dengue positive cases, majority *i.e.*, 211 (79.62) were below the age of 30 years. Similarly, majority *i.e.*, 184 (80.7%) of cases detected by NS1 and majority *i.e.*, 27(72.97%) of cases detected by IgM were in the age group <30 years. [Table-4] Association of dengue cases (detected by NS1) with thrombocytopenia was assessed as per the objective of the study. Thrombocytopenia was found in 162 (71.05%) of positive cases against only 152 (29.06%) of negatives and this difference was found to be statistically significant (p value <0.0001). [Table-4] shows association of dengue cases (detected by IgM) with thrombocytopenia. When IgM antibodies were considered for the diagnosis of dengue infection, thrombocytopenia was seen in 24 of 37(64.86%) positive cases and in only 216

(24.97%) of negatives and it was found to be statistically significant (p value= 0.01).
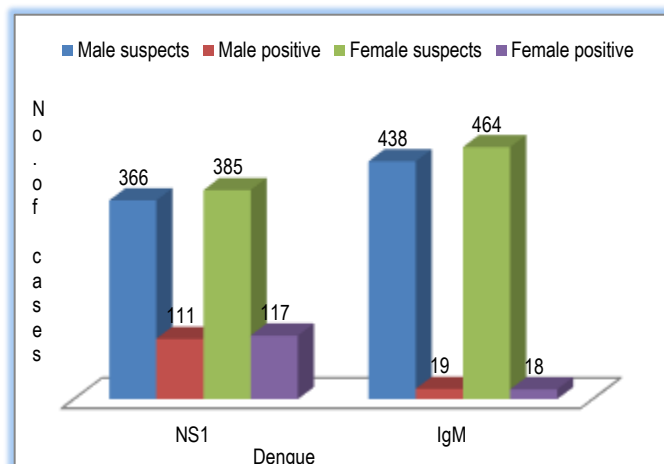

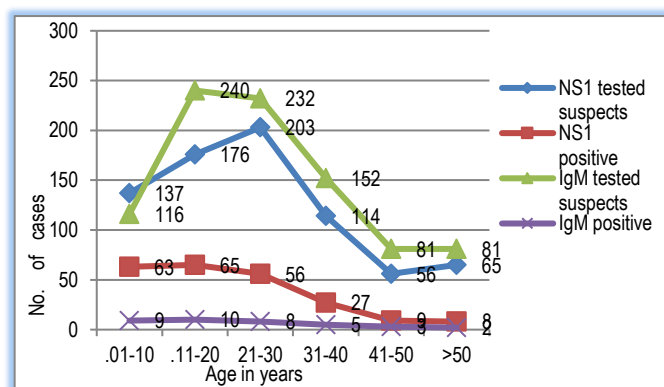Fig-1 Gender wise distribution of dengue suspects and positives

Fig-2 Age wise distribution of dengue suspects and positives

Table-1 Diagnosis of Dengue by dengue specific parameters

Parameters	Suspects tested	Positive	Rate of positivity
	No.	No.	(%)
NS1 only	751	228	30.35
IgM only	902	37	4.1
Total	1653	265	16.03

Table-4 Association of Dengue status (using NS1ag test) with thrombocytopenia

Thrombocytopenia	Dengue NS1 antigen				
	Positive		Negative		Total
	No.	%	No.	%	No.
Present	162	(71.05%)	152	(29.06%)	314
Absent	66	(28.94%)	371	(70.93%)	437
Total	228	(100%)	523	(100%)	751
$\varkappa^2$=115.06, p value <0.0001					

Table-5 Association of Dengue status (using Ig M test) with thrombocytopenia

Thrombocytopenia	IgM antibody				
	Positive		Negative		Total
Present	24	(64.86%)	216	(24.97%)	240
Absent	13	(35.13%)	649	(75.29%)	662
Total	37	(100%)	865	(100%)	902
$\varkappa^2$=6.359, p value= 0.01					

Discussion

Dengue is the most important arthropod-borne viral disease of public health significance for the past few decades. Early diagnosis of dengue is of utmost importance to reduce the morbidity and mortality associated with it, especially in the peripheries. Apart from dengue specific parameters, platelet count can be used in remote areas to support the diagnosis.

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Table-2 Gender wise distribution of Dengue suspects and positives

Gender	Total Suspects	Total Positive	Suspects tested for NS1	NS1 (positive)	Suspects tested for IgM	IgM (positive)
	No.	No (%)	No.	No (%)	No.	No (%)
Male	792	130 (49.05%)	366	111 (48.68%)	438	19 (51.35%)
Female	861	135 (50.94%)	385	117 (51.31%)	464	18 (48.64%)
Total	1653	265 (100%)	751	228 (100%)	902	37 (100%)

Table-3 Age wise distribution of Dengue suspects and positives

Age (Yrs)	Total Suspects	Total Positive	Suspects tested for NS1	NS1 (positive)	Suspects tested for IgM	IgM (positive)
	No.	No %	No.	No %	No.	No %
1-10	253	72 (27.16)	137	63 (27.63)	116	9 (24.32)
11-20	416	75 (28.3)	176	65 (28.5)	240	10 (27.02)
21-30	435	64 (24.15)	203	56 (24.56)	232	8 (21.62)
31-40	266	32 (12.07)	114	27 (11.84)	152	5 (13.51)
41- 50	137	12 (4.52)	56	9 (3.94)	81	3 (8.1)
51-60	146	10 (3.77)	65	8 (3.5)	81	2 (5.4)
Total	1653	265 (100)	751	228 (100)	902	37 (100)

The present study was undertaken to study the seroprevalence of dengue infection and to test association of dengue seromarkers with platelet count. In the present study out of 1653 clinically suspected dengue cases, 265 (16.03%) were found to be positive for dengue infection either by NS1 or IgM ELISA. Various studies have reported a seroprevalence level ranging from10%-21 % [2,15-18]. On the contrary, few other studies have found a lesser seroprevalence values [18,19] and some others have reported higher values than our study [8,21-24]. Of the 751 cases tested for NS1 antigen only, the rate of seropositivity was 228 (30.35%). This finding is consistent with the studies done by Kulkarni, *et al.,* [2] in which 30%, Agarwal, *et al.,* 29.41% [16], Neralwar, *et al.,* 29% [20], Mehta, *et al.,* 29.63% [21] and Datta, *et al.,* 23.3% [25] of cases were positive for NS1 antigen only. In contrast some studies have reported higher seropositivity for NS1 antigen [8,22,23] while other have reported lesser seropositivity [18,24,26]. On the other hand out of 902 tested for IgM only 37 (4.10%) cases were seropositive. Similar seropositivity rate was found in the study conducted by Gitanjali, *et al.* in which 4.7% [22], Sindhanai, *et al.,*10% [23], Lakshmi, *et al.,* 5% [24], patients were serologically positive for dengue infection. Higher values were found in other studies [2,8,16,18,20,21]. Of the 265 total positive cases of dengue, the highest numbers of cases were found in the age group of 1-30 years similar to the finding from other studies [2,20,27,28]. Comparatively lower immunity and intrinsically more permeable vascular endothelium in children render them more susceptible to dengue infection. Similarly, out of the total positive cases, 130 (49.05%) were males and the rest 135 (50.94%) were females contrary to the findings from other studies [18,20,21]. Thrombocytopenia in dengue infection is not an early indicator of severe disease but it helps in predicting the progression of disease and the present study tried to find the association of dengue positivity with thrombocytopenia. Thrombocytopenia was evident in 162 (71.05%) of positive cases as against only 152 (29.06%) of negative cases detected by NS1and it was in consistent with other studies [2,21-23]. Similarly, thrombocytopenia was more prevalent in positives *i.e.,* 24 of 37(64.86%) than in negatives 216 (24.97%) when diagnosed by IgM in the present study similar to the findings of other studies [2,21,23] The above results were statistically significant.

Conclusion
The present study highlights dengue infection rate in this part of state and its predominance in economically productive age groups. We found that thrombocytopenia was evident in dengue positive cases than in dengue negative cases.

Application of Research: Thrombocytopenia can be used as predictor of dengue infection for early initiation of therapy to reduce the morbidity and mortality associated with it, especially in poor resource settings. Such predictions also help to initiate preventive/ control measures well in time for the containment of spread of the disease.

Research Category: Diagnosis of Dengue

Abbreviations
IgM- Immunoglobulin M
Ig G- Immunoglobulin G
NS1- Non structural protein 1
CIMS- Chamarajanagar Institute of Medical Sciences
ELISA- Enzyme linked immunosorbent assay
DHF- Dengue hemmoraghic fever
DSS- Dengue shock syndrome
DV- Dengue virus
RT- PCR- Reverse transcriptase polymerase chain reaction
NASBA- Nucleic acid based amplification
WHO- World health organization
HRPO- Horse radish peroxidase
NIV- National institute of Virology
EDTA- Ethylene diamine tetra acetic acid

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