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A study on prevalence, an association of platelet count and seasonal distribution of Dengue from a tertiary care centre

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ABSTRACT

Dengue virus, transmitted through the bite of *Aedesaegypti* mosquitoes, is the causative agent of Dengue fever and Dengue shock syndrome. There are five serotypes of Dengue viruses identified so far. This study was undertaken to determine the prevalence of Dengue infection in a tertiary care hospital and to identify NS1Ag, IgM & IgG antibody depending on the duration of fever. This study is a prospective cross-sectional study conducted from January 2016 to February 2017. Sera samples received in the Microbiology Laboratory, from patients suspected to have Dengue by the clinicians; were tested for Dengue NS1 Antigen, IgM & IgG antibody using ELISA. The prevalence of Dengue was analysed about parameters like age, gender and seasonal changes. The positivity of NS1 Antigen, IgM and IgG Antibody in relation to the duration of fever was analysed. Among the 150 patients enrolled 18.7% (n=28) tested positive for Dengue. Among 28 laboratory-confirmed cases NS1 antigen was positive in 15(53.6%), IgM antibody positive in 5 (17.8%), IgG antibody positive in 4 (14.3%), NS1 Antigen and IgM positive in 1 (3.6%), IgM and IgG antibody positive in 2 (7.10%) and all three parameters NS1 antigen IgM and IgG positive in 1 (3.6%). From the serological tests, it was deduced that 20 cases had primary Dengue (IgM positive) and 8 had secondary Dengue (IgM and IgG positive). By clinical evaluation, Dengue fever was in 28 cases; four cases had hemorrhagic manifestations. No patients had evidence of Dengue Shock Syndrome. A number of Dengue cases were recorded in December 2016 (18%).

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INTRODUCTION

Dengue is one of the most important mosquito-borne viral infections affecting tropical and subtropical countries in the world. *Aedes aegypti* (Gupta N *et al.,* 2012) is the principal vector followed by *Aedesalbopictus.* These bite during the daytime. Man and Aedes are the principal reservoirs. Dengue fever presents with a nonspecific fever that mimics other viral/bacterial illnesses. Effective and accurate diagnosis of Dengue is of primary importance for clinical care, early detection of severe cases, case confirmation and differential diagnosis. Approximately 2.5 billion people live in Dengue-risk region with about 100 million new cases each year worldwide (Mustafa MS *et al.,* 2015).

Dengue viral infection is an emerging health problem, especially in South India (Gupta E, Ballani N 2014). Following the first outbreak in Kolkata in 1963-64, Dengue has spread to all parts of India, causing outbreaks periodically. Subsequently, recurring outbreaks of Dengue Fever (DF)/Dengue Haemorrhagic Fever (DHF) were reported in the

years 2005–2008 from various states in India.

India reported a total of 28,292 cases and 110 deaths in 2010, the highest number of cases and number deaths in a single year in the country in the previous two decades. All these have made India a Dengue hyperendemic area (Gupta E, Ballani N 2014). A recent outbreak occurred in Tamil Nadu in 2012, 2014 which took toll of many lives (Chandran R, Azeez PA 2015).

Dengue virus (DENV) is the most common arbovirus found in India. It has four serotypes (DEN-1 - DEN-4). Recently the fifth serotype (DEN-5) was discovered. Dengue virus belongs to the Flaviviridae family. DENV causes a broad spectrum of illness from mild asymptomatic illness to severe Dengue Hemorrhagic Fever / Dengue Shock Syndrome (DHF/DSS) (Gupta N *et al.,* 2012). The major diagnostic methods currently available are Real Time RT PCR and serological tests such as Immunoglobulin M (IgM) capture (MAC) ELISA (Shu P, Huang J 2004). At present NS1 antigen detection has been found to be more specific and efficacious than IgM detection in the first 4-5 days of illness. The efficacy of PCR and Dengue antibody combination has been found to be almost similar to the NS1 antigen, IgM and IgG Dengue antibody combination. Early diagnosis plays a crucial role in forecasting an early warning of an epidemic (Lateef *et al.,* 2018).

This study was carried out to find the prevalence of Dengue in our locality (throughout the year in relation to parameters like age, gender and seasons of the year) and also to compare and evaluate the detection rate of NS1 antigen assay, IgM MAC and IgG – ELISA, and analyse the status of platelet counts with different Dengue specific parameters as it is important when performed together in a single sample in relation to duration of fever (Azhar Omaran, 2017). Moreover, the knowledge and study gains additional importance given the Dengue outbreak in Tamil Nadu during August, September and October

Aim and objectives

1. To find out the prevalence of Dengue infection among the patients attending Saveetha Medical College.
2. To identify NS1Ag, IgM & IgG antibody using Enzyme-Linked Immunosorbent Assay (ELISA) depending on the duration of fever.
3. To distinguish whether the Dengue infections reported were primary infection or secondary infection
4. To correlate the platelet count with suspected cases of Dengue.

MATERIALS AND METHODS

A prospective descriptive study was undertaken in Saveetha Medical College on patients suspected with Dengue fever.

Study Subjects: Patients presenting with signs and symptoms of Dengue fever were enrolled, during the study period from January 2016 to February 2017. This study included both adult and pediatric patients who were enrolled asin-patients. Proforma was designed and the patient's details were collected accordingly. Proforma included details regarding name, age, sex, occupation and details about symptoms like fever, joint pains, body ache, maculopapular – rash, lymphadenopathy, hepato-splenomegaly, headache, abdominal symptoms, hemorrhagic manifestations etc. Proforma also included laboratory details such as the platelet count, haemoglobin, total count, differential count, MP& MF, Liver and renal function tests and radiological investigations such as CT/MRI/USG for selected cases.

Inclusion Criteria: Patients who had a fever of < 15 days duration with or without any of the following such as maculopapular rash, myalgia, headache, nausea, vomiting, joint pains, body pain and with or without petechiae were included.

Exclusion Criteria: Fever more than 15 days were excluded from the study. Those who were not willing for the study were not considered.

Ethical committee approval: The study was approved by the ethical committee (002/03/ 2016 /IEC/SU).

Methodology Dengue virus was tested using: Microwell ELISA test was done for the detection of Dengue NS1 Antigen in Human Serum/Plasma (J. Mitra & Co. Pvt. Ltd.) which has a sensitivity of 99.5% and specificity of 100%. MAC-ELISA test was performed for the detection of Dengue IgM Antibodies in Human Serum/Plasma (J. Mitra & Co. Pvt. Ltd.)which has a Sensitivity of 99.13% and Specificity of 99.84%. GAC- ELISA Test was performed for the detection of Dengue IgG Antibodies in Human Serum/Plasma (J. Mitra & Co. Pvt. Ltd.) which has a Sensitivity of 98.66% and Specificity of 99.93%. Then tests were performed strictly according to the manufacturer's instructions. The positivity of NS1 antigen, IgM and IgG antibody in relation to fever was analyzed.

RESULTS

A total of 117 adults and 33 paediatric patients were enrolled in the study during the period January 2016 to February 2017. The age of the patients ranged from 2 years to 60 years and above. The majority, i.e. 78% (n=117) of the adult

Table 1: Distribution of Dengue suspects in relation to gender

Gender	Total cases (n=150)	Percentage
MALE	78	52%
FEMALE	72	48%

Table 2: Distribution of Dengue suspects about age and gender

Age (Years)	Dengue suspects- Total n*	Dengue suspects-Male n*	Dengue suspects-Female n*	Lab-confirmed cases-Male(n)	Lab confirmed cases- Female(n)	Lab confirmed cases- Total n*
1-10	11(7.3)	2	9	1	4	5 (17.9)
11-20	22(14.7)	12	10	-	2	2 (7.1)
21-30	42 (28)	27	15	8	2	10 (35.7)
31-40	12 (8)	5	7	1	-	1 (3.6)
41-50	33 (22)	16	17	4	3	7 (25)
51-60	16 (10.7)	9	7	1	-	1 (3.6)
>60	14(9.3)	7	7	-	2	2 (7.1)
Total	150	78 (52)	72 (48)	15 (53.57)	13 (46.42)	28

* Figures in parenthesis indicate percentage

Table 3: NS1Ag, IgM & IgG ELISA positivity in relation to duration of fever

Duration of fever	Number of samples tested (n)	NS1 Antigen positive	IgM antibody positive n*	IgG antibody positive n*	NS1 & IgM antibody positive n*	IgM & IgG antibody positive n*	ALL 3 Positive	Total positives
<5 days	93	11(73.3)	0(0)	2(50)	1(100)	0(0)	0(0)	14
5-9 days	51	4(26.7)	4(80)	2(50)	0(0)	2(100)	1(100)	13
10-15 days	4	0(0)	1(20)	0(0)	0(0)	0(0)	0(0)	1
Total	150	15	5	4	1	2	1	28

*Figures in parenthesis indicate percentage

Table 4: Comparison of Platelet Count with Permutation Combinations of Dengue Parameters

Lab confirmed cases of dengue	Lab confirmed dengue cases	Platelet count	< 1 lakh
NS1 Antigen (+)	15	7	
IgM antibody (+)	5	2	
IgG antibody (+)	4	1	
NS1 & IgM antibody (+)	1	-	
IgM & IgG antibody (+)	2	2	
ALL 3 POSITIVE	1	1	
TOTAL	28	13 (46.4 %)	

patients with acute fever were in the age group of 18 to 50 years. 22% (n=33) of the paediatric patients with acute fever was less than eighteen years of age. The male to female ratio was found to be 1.1:1. The gender distribution of the study subjects is shown in Table 1. The patient's sera were analysed using ELISA tests for Dengue. among the 150 patients enrolled, 28 (18.7%) were found positive for Dengue. The distribution of Dengue suspects in relation to age and gender were depicted in Table:2 Lab confirmed cases was 5(17.9%), 2(7.1%), 10(35.7%), 1(3.6%), 7(25%), 1(3.6%) and 2(7.1%) in the age group 1-10, 11-20,21-30,31-40, 41-50,51-60, and>60 years respectively and the details are provided in Table :2

The distribution of NS1, IgM & IgG ELISA positivity of the lab confirmed Dengue cases are described in Graph:1. Among 28 laboratory-confirmed cases NS1 antigen was positive in 15(53.6%), IgM antibody positive in 5 (17.8%), IgG antibody positive in 4 (14.3%), NS1antigen and IgM positive in 1 (3.6%), IgM and IgG antibody positive in 2(7.10%) and all three parameters NS1 antigen IgM and IgG positive in 1 (3.6%). The positivity about the duration of fever, and status of NS1Ag, IgM and IgG ELISA are depicted in Table 3. NS1 Antigen was positive in 11(73.3%) patients who

Table 5: Clinical presentations of Dengue cases

Clinical Symptom/Sign	Age<20 years (n=33)*	21-40 years (n=54)*	Age 21-60 years (n=49)*	Age> 60 years (n=14)*
Fever	33(100)	54(100)	49(100)	14(100)
Headache	18(54.5)	37(68.5)	13(26.5)	10(71.4)
Vomiting	11(33.3)	10(18.5)	3(6.1)	3(21.4)
Retro-orbital pain	12(36.4)	19(35.2)	6(12.2)	3(21.4)
Muscle/Joint pain	14(42.4)	28(51.8)	11 (32.4)	9(64.3)
Weakness	16(48.5)	40(74.1)	14(28.6)	10(71.4)
Abdominal pain	14(42.4)	7(12.9)	2(4.1)	2(14.3)
Hepatomegaly	10(30.3)	28(51.9)	1(2)	3(21.4)
Mucosal bleeding	2(6)	2(3.7)	1(2)	1(7.1)
Elevated liver enzymes$	11(33.3)	10(18.5)	2(4.1)	2(14.3)
Low platelet count$	4(12.1)	6(11.1)	2(4.1)	1(7.1)

$- Investigations; *-Figures in parenthesis indicate the percentage

presented with fever less than 5 days duration. IgM positivity was noted in cases presented with clinical signs and symptoms of Dengue after 5days, whereas IgG was positive in two cases even within five days of fever.

The analysed platelet count of patients with Dengue positive cases is given in Table 4. It was noticed that seven (63.6%) had platelet count between 50,000-1 lakh cells /mm (Dengue, 2017). Three patients had platelet count less than 50,000. From the serological tests, it was deduced that 25 cases had primary Dengue (IgM positive) and 3 had secondary Dengue (IgM and IgG positive). By clinical evaluation, Dengue fever was seen in 28 cases. Among them, four cases had hemorrhagic manifestations. No patients had evidence of Dengue Shock Syndrome. The mortality rate was nil during the study period due to timely diagnosis and management.

Maximum number 10 of 28; of Dengue suspects were observed in the age group 21-30. The dengue suspects were more among males (52%) than in females (48%). The prevalence of Dengue among the 150 Dengue suspects in our study was observed to be 18.7%, i.e. 28 samples were positive by ELISA either by NS1 antigen detection, IgM and IgG antibody detection or all three. The lab confirmed Dengue cases were predominant in the 21-30 age group (27%) followed by the 31-40 age group (21.9%). The lab confirmed dengue cases were more in males (53.6%) than females (46.4%), which was statistically significant (P<0.05). Graph 2. Depicts the distribution of Dengue suspected during the study period and, the prevalence was more in December and January following the rains after which there was a decline in Dengue fever cases.

Some Dengue cases were recorded in December 2016 (18%), followed by January 2016 and November 2016 (12%). (10%) in October 2016, (9.3%) in January 2017 and (8%) in September 2016, followed by 2016 (7%) in August 2016. The highest peak of Dengue incidence is found to occur from September to December and it was low in April and none in May during the study periods. The occurrence of Dengue cases is comparatively lower during the other months of the year. Details of the clinical presentations of the cases enrolled for the study are provided in Table: 5. Fever was seen in all patients of all age groups. The common manifestations were a headache, weakness, abdominal pain and myalgia followed by other symptoms and signs like retro-orbital pain, hepatomegaly. Elevated liver enzymes and low platelet count were seen in patients respectively.

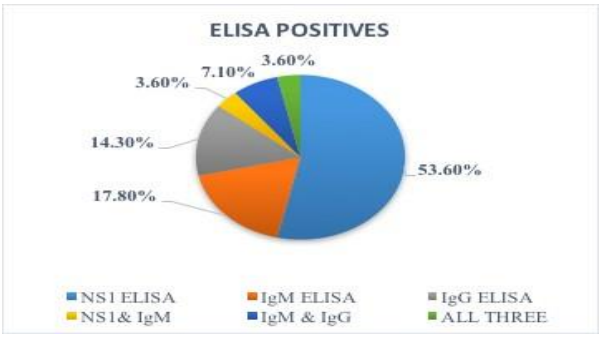

Figure 1: Distribution of NS1, IgM & IgG ELISA positivity of the lab confirmed Dengue cases

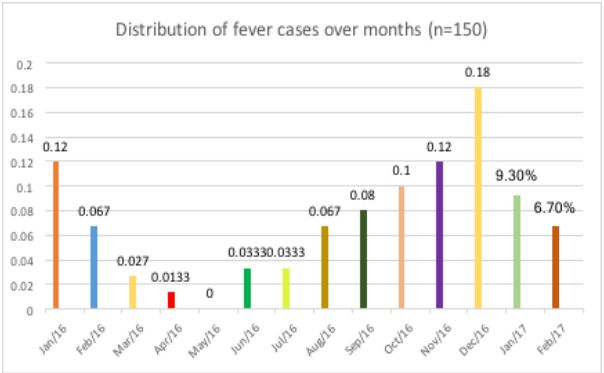

Figure 2: Distribution of Dengue suspect cases during the study period (Seasonal variation)

DISCUSSION

This study was carried out to analyze the prevalence of Dengue in a tertiary care rural teaching hospital, in relation to parameters like age, gender, platelet count and seasonal variations during the study period and to find out the efficacy of detection of Dengue cases by combined NS1 antigen, IgM and IgG antibody testing in relation to duration of fever as this type of study has not been done in this area.

The prevalence of dengue among the 150 suspects in our study was observed to be 18.7% (n=28) tested positive for Dengue. Positive by ELISA either by NS1 antigen detection, IgM and IgG antibody detection, both or all three. The lab confirmed dengue cases were predominant in the 21-30 age group (28%) followed by the 41-50 age group (22%). Such cases were noticed more in males (52%) when compared to females (48%). This finding is in concordance with the study by (Bhaswathi B *et al.,* 2012) where the highest number of Dengue cases was from the 11-30 age group and males were predominantly affected and the finding was discordant with (Garg *et al.,* 2011) where highest Dengue cases were from the pediatric population.

Fever was seen in all patients of all age groups. In the <20 age group, the common manifestations were a headache (54.5%), weakness (48.5%), abdominal pain (42.4%) and myalgia (42.4%) followed by other symptoms and signs like retro-orbital pain, hepatomegaly. Elevated liver enzymes and low platelet count were seen in 33.3% and 12.1% of patients respectively. These findings are concordant with the study by (Mishra *et al.,* 2016) were fever and headache were the predominant manifestations in children. Hemorrhagic and bleeding tendency was seen more in patients less than 20 years (6%) when compared to the adults (3.7%) and elderly (2%). This is in concordance with the study by (Mishra *et al.,* 2016). These clinical manifestations can be helpful in making a presumptive diagnosis of Dengue and guide us to take steps to confirm the diagnosis and provide appropriate care.

Among 28, laboratory-confirmed cases NS1 antigen was positive in 15(53.6%), IgM antibody positive in 5 (17.8%), IgG antibody positive in 4 (14.3%), NS1antigen and IgM positive in 1 (3.6%), IgM and IgG antibody positive in 2(7.10%) and all three parameters NS1 antigen IgM and IgG positive in 1 (3.6%). The positivity was related to the duration of fever.

Among the 5 samples that were positive only for IgM, were from patients with fever for 5-9 days. No sample from patients with fever for less than 5 days was positive for IgM alone. All the 15 samples that were positive for NS1 alone had the fever for less than 5 days. One sample was positive for both NS1 antigen and IgM antibody, and he had a fever of four days. Another case who was positive for both IgM and IgG antibody had a fever for seven days. Similarly, one another case who was positive for NS1 antigen, IgM and IgG antibody had the fever of eight days before evaluation.

Both NS1 antigen and IgM antibody were mostly positive between 4 – 7 days of illness according to a study by (Kushal *et al.,* 2015). In a study by (Kwoon *et al.,* 2010), Dengue NS1 antigen assay was found to be 87% sensitive in picking up Dengue cases in the first 3 days of fever. A study by (Fauziah *et al.,* 2011) has shown Dengue antigen and antibody tests to have the highest detection rate between days 3 and 4 days of fever. The antibodies were detected more compared to antigen between days 5 and 6 according to their study, which is slightly discordant with our study results. After day 10, the detection rate decreased significantly for both antigen and antibody.

Thus, for fever less than 5 days, the ideal test is NS1 antigen detection. For 5-9 days fever, both NS1 antigen and IgM antibody detection can be done. In fever for more than 10 days IgM antibody detection is ideal and NS1 antigen detection is of less value. From all these details, it can be drawn that the ideal time for Dengue detection is between 5 and 10 days post onset of symptoms, as both NS1 antigen and IgM antibody can be detected, which can help the practitioner make a reasonable diagnosis. The elevated IgM observed in a sample could be the result of an infection that occurred 2 to 3 months ago.

In addition, there is cross-reactivity with other filoviruses including West Nile virus (WNV), St. Louis encephalitis virus (SLE), Japanese encephalitis virus (JEV) and yellow fever virus (YFV). The NS1 antigen is highly specific for Dengue. NS1 antigen remains elevated from the first day of fever up to the tenth day in case of primary Dengue and CDC has documented NS1 antigen detection even up to eighteen days post onset of symptoms. However, in case of secondary Dengue, NS1 becomes undetectable earlier than in primary Dengue. So, detection of both the NS1antigen and IgM antibody between 5-10 days of fever is more valuable in the diagnosis of Dengue than detecting either of the two. False positives and false negatives can also be prevented to a greater extent by detecting both antigen and antibody.

Platelet count approximately goes below 25000 in 30%, lies between 25000 and 50000 in another 30%, and above 50000 but below 100000 in other

30%. In about 10% of cases, it was above 100000. The overall reduction in platelet count was noticed in Dengue cases more in children and females. Some Dengue cases were recorded in December 2016 (18%), followed by January 2016 and November 2016 (12%). (10%) in October 2016, (9.3%) in January 2017 and (8%) in September 2016, followed by 2016(7%) in August 2016. In the present study, the highest peak of Dengue was found during September, October, November and December and a relatively lowest in April and no cases during May. All these are in agreement with the study by (Gupta *et al.,* 2014). These findings are concordant with the study results of (Garg *et al.,* 2013).

The monsoon periods in Tamil Nadu are from June to September (32% of annual rainfall) and October to December (48% of annual rainfall). Stagnant water and damp conditions following the monsoon season are ideal for the virus to grow and multiply. This might be the reason for the sharp spike in Dengue cases following rainfalls, which explains the reason for the spike of cases from September to December, as documented in our study. These were conclusions which were drawn in a study by (Chandran *et al.,* 2015), which explains the results of our study. In addition to that, the rise in average temperature, an element of climate change, favours higher breeding and spread of the vectors such as *Aedesaegypti*, and consequently spread of Dengue virus. This can explain the reason for cases seen during the dry months in our study period.

Limitations: The study was limited to a single centre and carried out with a small number of cases

Future directions: Large number cases have to be studied in multiple centres with consistent methods to find out the variations in presentation and immunological response, susceptibility, clinical course and outcome or complications, and changing trends. To design and prepare regional guidelines, to enable the practitioners to handle cases of Dengue and the complications associated with it, and report them to the public health authorities.

CONCLUSION

To conclude community education on the awareness of the symptoms of Dengue, the pattern of Dengue, mechanism of spread, seasonal variation and preventive measures through audio-visual means to a great extent likely help in achieving Dengue control. Control of vector breeding sites by appropriate measures and community health education will prevent Dengue outbreaks in near and distant future.

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