Seroprevalence of Dengue cases detected by ELISA technique in Bharatpur District of Rajasthan

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Abstract: Dengue is a common febrile arthropod borne viral disease caused by any of the four serotypes of dengue virus. It has wide spectrum of clinical presentation and often has unpredictable clinical evolution and outcome. Approximately 50 million infections occur annually world-wide. An increase in the number of cases of dengue has been noticed in India during recent years. Present study was carried out to determine seropositivity, clinical profile and seasonal variation of dengue infection in Bharatpur district of Rajasthan, India. Study was carried out from suspected dengue patients attending Government RBM hospital during the period January to December 2017. Blood samples were collected from 1167 patients with dengue like illness and serum separated. All samples were tested for both Dengue NS1 antigen & IgM antibody ELISA. Prevalence of dengue NS1 Ag ELISA 157/717 (21.90%) samples were positive and for dengue IgM Ab ELISA 81/450 (18.00%) samples were positive. The total prevalence of dengue was 238/1167 (20.40%) in the district. Total number of positive case (NS1 antigen and IgM antibody ELISA inclusive) shows that maximum patient were in 1 to 30 year age group. Males were more affected (22.90%) than females (15.95%). The highest numbers of samples were collected during rainy season that is from September to November and highest positivity of dengue virus had been reported during this period. Number of dengue cases in Bharatpur district showed a gradual increase from August to October. The disease started spreading in August, peaked in October and slowly tapered by December.
Keywords: Dengue, Seropositivity, Dengue Hemorrhagic Fever, Dengue Shock Syndrome, ELISA.

INTRODUCTION

Dengue is a common febrile arthropod borne viral illness caused by a single stranded enveloped RNA virus belonging to the family Flaviviridae, genus Flavivirus [1]. Dengue infection is caused by any one of four distinctive antigenically related dengue virus serotypes: DENV-1, DENV-2, DENV-3 and DENV-4 [2]. In India 60 lakhs of dengue cases were reported by lab diagnosed. Whereas the No. of cases official reported are very less in compression. This data has been collected between 2006 and 2017 [3]. Dengue fever is a self limiting disease becoming dangerous and deadly public health problem due to lack of early diagnosis of the disease in the early and acute phase of illness. This lack of early treatment may result in mortality of the patient suffering from dengue fever with complications like DHF (Dengue Haemorrhagic Fever)/DSS (Dengue Shock Syndrome) [4]. The incidence of dengue has grown dramatically around the world in recent decades. Over 2.5 billion people – over 40% of the world's population are now at risk from dengue. WHO currently estimates there may be 50–100 million dengue infections worldwide every year [5]. In India, a dengue infection has been frequently encountered in epidemic proportions in several states [6,7]. There is no specific treatment for dengue, but early detection and proper medical care may decrease the mortality from it.

As effective control and preventive programmes for dengue infection are based upon proper surveillance data and its analysis, our study was done to know the trend and prevalence of dengue virus infection during one year period from January 2017 to December 2017.

MATERIALS AND METHODS

A total of 1167 blood samples from clinically suspected dengue patients attending Government RBM

hospital during the period January to December 2017 at Bharatpur district of Rajasthan.

Inclusion Criteria

Outdoor and indoor patients with febrile illness having symptom such as joint pain, rash, myalgia, Retro-orbital pain, severe headache and haemorrhagic manifestation were included in the study.

Patients having history of fever for less 5 days were tested for NS1 Antigen by ELISA technique and those with fever for more than 5 days were tested for IgM Antibody by ELISA technique, Blood samples were collected and serum was separated as per the standard guidelines [8,9]. Blood collection tubes were kept upright until the blood clotted. Clotted blood was centrifuged to separated serum and tests were performed on this separate serum.

The serum samples were subjected to dengue NS1 antigen and IgM antibody detection with ELISA

kit manufactured by J. Mitra & Co. Pvt. Ltd. The tests were performed according to manufacturer's instruction. Test for dengue NS1 Ag are based upon the principle of "Direct Sandwich" ELISA. The microwells are coated with Anti-dengue NS1 antibodies with high reactivity for Dengue NS1 Ag. Test for dengue IgM Ab are based upon the Principle of an enzyme immunoassay based on "MAC Capture ELISA". Anti human IgM antibodies are coated on to microtiter wells.

RESULTS

The total 1167 serum samples from suspected dengue cases were collected. In these 717 samples were tested for NS1 Ag ELISA and rest 450 samples were tested for IgM Ab ELISA.

Out of the total samples tested for NS1 Ag ELISA 157 (21.90%) samples were positive and for dengue IgM Ab ELISA 81 (18.00%) samples were positive (table-1). The total prevalence of dengue was 238 (20.40%) in the district.

Table-1: Positivity of dengue by NS1 Ag and IgM Ab ELISA

Results	NS1 Ag ELISA (n=717) (%)	IgM Ab ELISA (n= 450) (%)
Positive	157 (21.90)	81 (18.00)
Negative	560 (78.10)	369 (82.00)
Total	717 (61.44)	450 (38.56)

The patients enrolled in our study were between 1-90 years of age. The distribution of the patients as per age is given in table 2. Maximum numbers of patients found positive were in the age group 1-10 year i.e. 374 (32.05%), followed by 11-20

year 225 (19.28%), 21-30 year 185 (15.85%), 31-40 year 137 (11.74%), 41-50 year 86 (7.37%), 51-60 year 64 (5.48%), 61-70 year 55 (4.71%), 71-80 year 25 (2.14%), 81-90 year 16 (1.37%).

Table-2: Age wise distribution of patient blood samples collected in the study

Age in year	Number of patients (%)
1-10	374 (32.05)
11-20	225 (19.28)
21-30	185 (15.85)
31-40	137 (11.74)
41-50	86 (7.37)
51-60	64 (5.48)
61-70	55 (4.71)
71-80	25 (2.14)
81-90	16 (1.37)
Total	1167 (100)

Age-wise distribution of dengue positive patients of varying age group is given in Table 3. Total number of positive case (NS1 ELISA and IgM ELISA inclusive) shows that maximum patient were in 1 to 30 year age group. Higher positivity of dengue virus was

found in age groups 11-20 years (38, 24.20% NS1 & 13, 16.05% IgM) followed by 1-10 years (35, 22.30% NS1 & 34, 41.97% IgM) and 21-30 years (25, 15.92% NS1 & 12, 14.81% IgM).

Table-3: Age wise distribution of dengue NS1 Ag and IgM Ab ELISA positive cases

Age in year	NS1 Ag ELISA		IgM Ab ELISA	
	Positive (%)	Negative (%)	Positive (%)	Negative (%)
1-10	35 (22.30)	153 (27.32)	34 (41.97)	152 (41.20)
11-20	38 (24.20)	124 (22.14)	13 (16.05)	50 (13.55)
21-30	25 (15.92)	98 (17.50)	12 (14.81)	50 (13.55)
31-40	21 (13.38)	61 (10.90)	5 (6.17)	50 (13.55)
41-50	16 (10.20)	45 (8.04)	6 (7.41)	19 (5.15)
51-60	8 (5.10)	35 (6.25)	4 (4.93)	17 (4.61)
61-70	6 (3.82)	24 (4.30)	3 (3.70)	22 (5.96)
71-80	5 (3.20)	12 (2.14)	3 (3.70)	5 (1.35)
81-90	3 (2.00)	8 (1.42)	1 (1.23)	4 (1.08)
Total	**157 (100)**	**560 (100)**	**81 (100)**	**369 (100)**

Table-4: Month wise distribution of dengue NS1 Ag and IgM Ab ELISA positive cases

Month (2017)	NS1 Ag ELISA			IgM Ab ELISA		
	Samples tested (%)	Positive (%)	Negative (%)	Samples tested (%)	Positive (%)	Negative (%)
Jan	16 (2.23)	3 (18.75)	13 (81.25)	9 (2.00)	2 (22.22)	7 (77.78)
Feb	10 (1.40)	2 (20.00)	8 (80.00)	4 (0.89)	1 (25.00)	3 (75.00)
Mar	8 (1.11)	2 (25.00)	6 (75.00)	6 (1.33)	1 (16.67)	5 (83.33)
Apr	10 (1.40)	3 (30.00)	7 (70.00)	6 (1.33)	4 (66.67)	2 (33.33)
May	31 (4.32)	6 (19.35)	25 (80.65)	24 (5.33)	3 (12.50)	21 (87.50)
Jun	36 (5.02)	4 (11.11)	32 (88.89)	28 (6.22)	1 (3.57)	27 (96.43)
July	44 (6.14)	2 (4.55)	42 (95.45)	42 (9.33)	4 (9.52)	38 (90.48)
Aug	71 (9.90)	20 (28.17)	51 (71.83)	66 (14.67)	7 (10.60)	59 (89.40)
Sep	138 (19.25)	31 (22.46)	107 (77.54)	83 (18.44)	14 (16.90)	69 (83.10)
Oct	172 (24.00)	38 (22.10)	134 (77.90)	83 (18.44)	17 (20.48)	66 (79.52)
Nov	148 (20.64)	45 (30.41)	103 (69.59)	77 (17.11)	21 (27.30)	56 (72.70)
Dec	33 (4.60)	1 (3.03)	32 (96.97)	22 (4.89)	6 (27.30)	16 (72.70)
Total	**717 (100)**	**157 (21.90)**	**560 (78.10)**	**450 (100)**	**81 (18.00)**	**369 (82.00)**

The above table shows that the highest number of samples were collected during rainy season that is from September to November and highest positivity of dengue virus had been reported during this period.
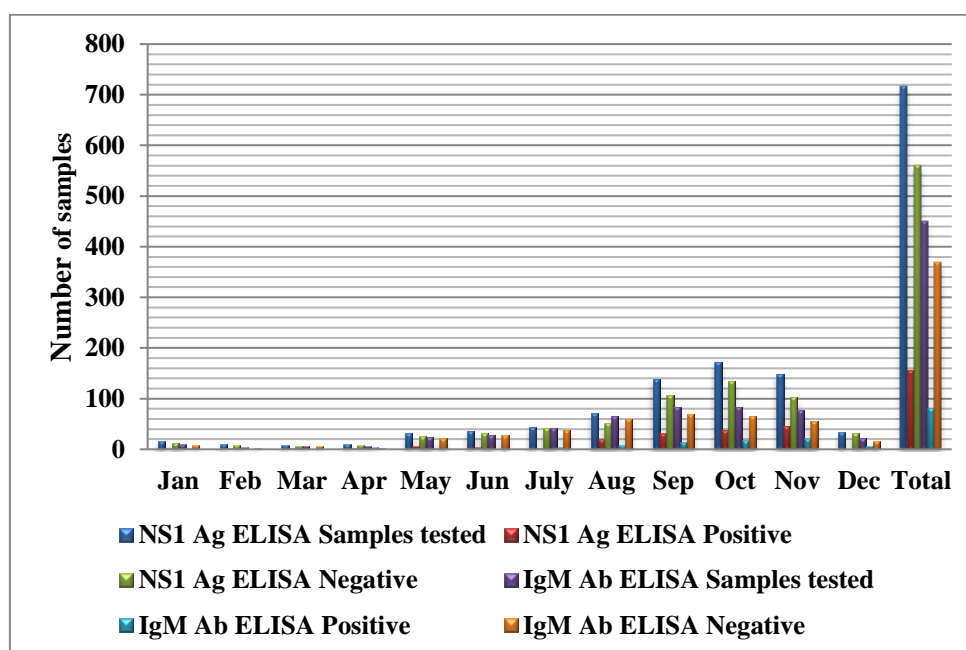

Fig-1: Bar diagram showing distribution of Dengue positivity from Jan – Dec 2017

Table-5: Sex wise distribution of dengue NS1 Ag and IgM Ab ELISA positive cases

Sex	NS1 Ag ELISA		IgM Ab ELISA	
	Positive (%)	Negative (%)	Positive (%)	Negative (%)
Male	112 (71.33)	362 (64.64)	59 (72.83)	214 (58.00)
Female	45 (28.67)	198 (35.36)	22 (27.17)	155 (42.00)
Total	**157 (100)**	**560 (100)**	**81 (100)**	**369 (100)**

A total of 171 positive samples of male patients which included both NS1 Ag 112 (71.33%), IgM Ab 59 (72.83%) tested by ELISA and a total of 67 positive samples of female patients which included both NS1 Ag 45 (28.67%), IgM Ab 22 (27.17%) tested by ELISA showed that males were more affected (22.90%) than females (15.95%) by dengue virus.

Table-6: Total dengue positive cases detected by both NS1 Ag and IgM Ab ELISA in Males and Females

Sex	Positive (%)	Negative (%)	Total (%)
Male	171 (22.90)	576 (77.10)	747 (100)
Female	67 (15.95)	353 (84.05)	420 (100)
Total	**238 (20.30)**	**929 (79.70)**	**1167 (100)**

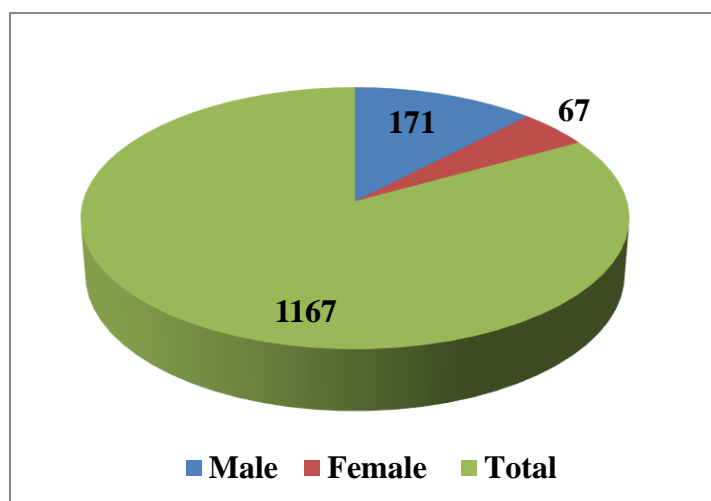Overall positivity was more in males 22.90% than females 15.95%.

Fig-2: Pie chart for positivity of dengue virus in Males and Females

DISCUSSION

WHO has declared dengue to be major endemic disease in India and cyclical epidemic outbreaks are becoming more frequent. The public data of reported cases of dengue fever are inadequate. There has been a more increase in number of dengue cases every year and mortality rate has been down steadily as well [8].

In our study, out of 1167 dengue suspected cases, 157 (21.90%) patients were positive for dengue NS1 Ag and 81 (18.00%) patients were positive for dengue IgM Ab by ELISA. The overall prevalence of both ELISA in dengue suspected patient during our study was found to be 238 (20.30%). Sood S reported 18.99% seroprevalence of dengue in Rajasthan, India [9]. Deshkar ST *et al.* reported the prevalence of dengue positivity of 24.49% which is closely related to our study [10]. Ukey PM *et al.* reported, the higher seroprevalence of dengue 31.3% in central India [11].

Saini S *et al.* reported seropositivity of 30.6% in western Maharashtra [8], Seropositivity of 17.7% was reported by Rao MS et al in Andra Pradesh, India [12].

In the present study, out of 238 (both NS1 Ag & IgM Ab ELISA) positive cases, maximum positivity was found in 1 to 30 years. Highest positivity of dengue virus was found in age groups 11-20 years (38, 24.20% NS1) & (13, 16.05% IgM) followed by 1-10 years (35, 22.30% NS1) & (34, 41.97% IgM) and 21-30 years (25, 15.92% NS1) & (12, 14.81% IgM). This was comparable to other studies of Gore MM, Baruah [13] and Dash PK *et al.* [14]. In another study, Deshkar ST *et al.* reported out of 3,822 MAC ELISA positive cases, 40.50% cases were in the age group of 0-10 years, followed by 26.71% cases in the age group of 11-20 years [10]. Rao MS *et al.* also observed maximum seropositivity of 35.84% in the age group of 0- 10 years, followed by 22.66% in the age group of 11-20 years [12]. Ukey PM *et al.* reported highest

seropositivity of 43.90% in children < 10 years followed by 31.71% in age group of 15-30 years [11]. The high number of cases in the paediatrics and young adult age group implies that the disease is endemic in these regions. In these areas, adults manifest with disease less, as they possess better immunity as compared to small children.

Total 1167 samples were studied out of which 747 patients are male and 420 patients of female. Data showed that male patients are more infected than female patients. We observed a total of 171 positive samples of male patients which included both NS1 Ag 112 (71.33%), IgM Ab 59 (72.83%) tested by ELISA and in a total of 67 positive samples of female patients which included both NS1 Ag 45 (28.67%), IgM Ab 22 (27.17%) tested by ELISA it was concluded that males were more affected 22.90% than females 15.95% by dengue virus. Karoli R *et al.* reported 58% male patients and 42% females with M: F of 1.38: 1 [15]. Male to female ratio of 1.82:1 was reported in another study [16]. However, approximately equal number of affected females 550 (50.70%) and males 535 (49.30%) were reported by Murugananthan K *et al.* [17]. In our study we also found that males were more affected than females by dengue febrile illness. Higher seropositivity in males might be because of increased exposure at work places or outdoor activities.

To identify the seasonal variation of the disease, analysis of the data on monthly basis was done. The infection started spreading in August, peaked in October and slowly tapered by December. Similar findings were reported by Kumar A *et al.,* who observed a gradual increase in cases from June with a peak in September, during of the study [16]. Another similar finding also reported maximum positivity of dengue in the months of August, September, October and November [10]. Gunasekaran P *et al.* also reported high percentage of IgM positivity during the months of September and October [18].

Aedes aegypti has an average adult survival of fifteen days. During the rainy season, survival is longer and therefore the risk of virus transmission is greater. During post monsoon period, stagnant water pool collected during rainy season acts as favourable breeding sites along with lower temperature during this period, there is an increase in transmission of dengue infection [19, 20].

The seasonality of transmission of dengue with increased activity in monsoon and post monsoon season was seen in the present study in accordance with the reported patterns of dengue transmission by Gupta *et al.* [7].

CONCLUSION
Number of dengue cases in Bharatpur district showed a gradual increase from August to October. The disease started spreading in August, peaked in October and slowly tapered by December. The prevalence of positive cases of dengue was found to be more in males than females.

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