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Original Article

Microbiology Section

Trend of Dengue Virus Infection with Seasonal Variation at HIMS, Varanasi, Uttar Pradesh, India

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ABSTRACT

Introduction: Dengue is the most common mosquito borne arboviral infection in India, and it has become endemic in India with consistent episodes. Dengue is an acute febrile illness caused by Dengue Virus (DENV-1, DENV-2, DENV-3, DENV-4), an arthropod of family Flaviviridae, transmitted through the bite of female *Aedes aegypti* mosquitoes. Although, dengue is more prevalent in humans but also it may sometimes be seen in monkeys. The only animals to be affected when bitten by a mosquito, infected with dengue fever are monkeys other animals do not carry or spread dengue. Patient presents with hyperthermia, headache, severe joint and muscle pain, fatigue and skin rashes. Neurological manifestation in sever dengue results from multiorgan dysfunction due to cerebral edema, haemorrhage due to vascular leak, cerebral hypoperfusion, and electrolyte disturbances.

Aim: To study serological markers with association of platelet count and trend of DENV infection with seasonal variation.

Materials and Methods: The present retrospective study was conducted for a period of two years in Heritage Institute of Medical Sciences (HIMS), Varanasi, Uttar Pradesh, India, from January 2017 to December 2018. Blood samples were collected for 2140 suspected dengue patients. Out of the serologically positive cases, serological confirmation and platelet count was done for dengue infection. Data was presented as percentages.

Results: Total 2140 suspected cases were admitted and out of them 199 (9.3%) were found to be seropositive for dengue infection. Non structural protein 1 (NS1) positive cases out of which total seropositive cases were reported to be 127 (63.81%) and 147 (73.86%) cases were positive for NS1 antigen either alone or along with antibody. Out of the total 199 positive cases thrombocytopenia was observed in 126 (63.31%) cases. Positive cases which were under 15 years of age were reported to be 92 (46.23%).

Conclusion: The study concludes that NS1 antigen and IgM-IgG antibody consideration in the diagnosis of dengue infection builds the opportunity of early diagnosis so as to keep away the complications significantly.

Keywords: Mosquito borne arboviral infection, Non structural protein 1 antigen, Seropositivity, Viral infection

INTRODUCTION

Dengue is an acute febrile illness endemic to the Indian sub-continent. It is caused by DENV (DENV)-an arthropod-borne virus of the family *Flaviviridae* and is transmitted to humans by *Aedes* mosquitoes, mainly *Aedes aegypti*. Four distinct serotypes have been described for DENV-Serotypes-1-4 [1,2].

Based on neutralisation assay data, four serotypes (DENV-1, DENV-2, DENV-3, and DENV-4) can be distinguished [2]. Dengue characteristic features are- fever, severe headache, muscle and joint pain, nausea, vomiting, eye pain and rash. A severe form of the disease, Dengue Haemorrhagic Fever (DHF)/Dengue Shock Syndrome (DSS) principally affects children [3-6].

Currently, the three basic methods used by most laboratories for the diagnosis of DENV infection are virus detection, detection of the viral Ribonucleic Acid (RNA) by Reverse Transcription Polymerase Chain Reaction (RT-PCR) and demonstration of DENV-specific circulatory antibodies by the IgM capture Enzyme-Linked Immunosorbent Assay (MAC-ELISA) and/or the rapid dengue strip Immnunochromatoghraphic Test (ICT). Rapid and sensitive laboratory methods are required for early detection of the disease to reduce the morbidity and mortality [7].

All four DENV serotypes are capable of causing dengue fever, with the induction of an immune response that in most cases leads to lifelong protection against clinical disease arising from infection with the homologous serotype. Secondary infection with a serotype different from that causing primary infection may lead to DHF or DSS [1].

Up to 20 million people are infected globally each year [3]. Infection with DENV can result in a relatively benign, acute febrile illness (dengue fever) or in severe disease with abnormalities in vascular permeability DHF which can sometimes lead to sudden and often fatal hypovolemic shock DSS [3].

The DENV infection is a significant reason for malady in tropical and subtropical zones, with an estimated 50 million infections occurring every year and more than 2.5 billion individuals being in danger of infection [3]. The risk of the DHF is about 0.2% during the first dengue infection but is at least 10 fold higher during infection with a second DENV serotype. The fatality rate with DHF can reach 15% but can be reduced to less than 1% with proper treatment [7]. IgM antibodies in dengue infection appear in early three days of viral fever and lasts for 30-60 days, whereas IgG appears around seven day, reaches peak at 2-3 weeks and persists the entire life [8-14]. Concurrent evaluation for the NS1 antigen alongside testing for IgM and IgG class antibodies to DV (DENGM) provides optimal diagnostic potential for both early and late dengue disease [11]. The aim of this study was to know the changing trend due to seasonal variation in dengue infected cases, also to determine seroprevalence of DENV and to associate the platelet count with its serological markers.

MATERIALS AND METHODS

The present study was conducted retrospectively over a period of two years from January 2017-December 2018 and data was collected and analysed over a period of three months (October 2018-December 2018) during same period. The study was conducted in the Department of Microbiology, Heritage Institute of Medical Sciences, Varanasi. Approval from Ethical Committee was obtained (Ref No. HIMS/IEC/025).

A total of 2140 cases were included who attended the Outpatient Department (OPD), Inpatient Department (IPD) and Emergency clinic at HIMS, Varanasi, Uttar Pradesh, India.

Data Collection

Serum samples were collected from all the suspected patients whose clinical features were similar to dengue infection. Data was analysed on the basis of age, sex, seasonal distribution of all positive cases with serology and platelet count evaluation was done. In this study J.MITRA (dengue day 1 test) immunochromatographic card kit was used, this immunochromatographic card is based on principle of antigen-antibody reaction. This immunochromatographic card was used for the qualitative detection of NS1 antigen and differential IgM and IgG antibody in the serum sample. Dengue NS1 antigen device two line; "C" (Control line) and "T" (Dengue NS1 antigen test line). Test line was coated with anti-dengue NS1 Ag. The sample was to the device, Dengue NS1 antigen present in the sample reacted to the anti-dengue NS1 gold colloid conjugated making antigen-antibody complex. This complex migrated along the membrane to the test region and formed the visible pink line at "T" as antigen-antibody gold conjugate complex. Dengue IgM/IgG test device consisted of three lines; "C" (Control line), "M" (IgM test line) and "G" (IgG test line). IgM and IgG test line are coated with anti-human IgM and IgG monoclonal antibodies respectively. When sample was added to the device, IgM and IgG antibodies in the sample reacted with anti-human IgM and IgG antibodies coated on the membrane. Colloidal gold complexes containing dengue 1-4 antigen prepared from DENV culture was captured by the bound anti-dengue IgM or IgG on respective test band located in the test window causing a pale to dark red band to form at the IgG or IgM region of the test device window.

STATISTICAL ANALYSIS

Descriptive analysis was done and data was presented as numbers and percentages were calculated.

RESULTS

Total number of patients tested (from January 2017 to December 2018) for suspected dengue infection were 2140, out of them 199 (9.3%) cases were positive for dengue and 1941 (90.7%) cases were negative. Out of 199 cases, 136 (68.34%) positive cases were of male and 63 (31.66%) positive cases were of female patients and most infected age group for dengue infection was 0-15 years male and female [Table/Fig-1].

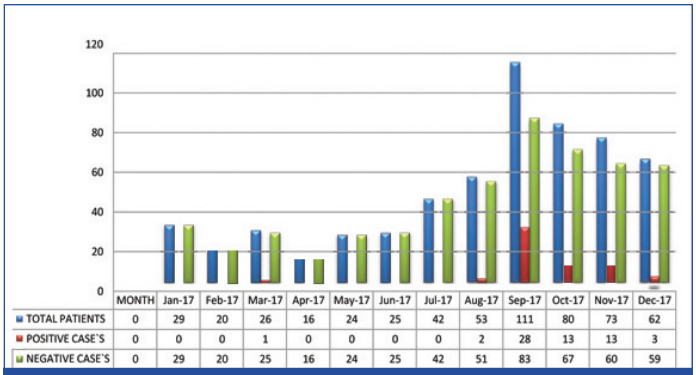
Age group (years)	Male patients	Female patients	Total
0-15	66	26	92 (46.23%)
16-30	34	20	54 (27.14%)
31-60	27	14	41 (20.60%)
>60	9	3	12 (6.03%)
Total	136 (68.34%)	63 (31.66%)	199 (100%)

[Table/Fig-1]: Age and sex wise distribution of dengue patients.

[Table/Fig-2,3] shows yearly distribution of cases in 2017 and in 2018, in January 2018 to April 2018, two positive cases were found; in the period of May 2018 to August 2018 only one positive case was found, corresponding to 2017 data most of the positive cases (136) were in September 2018 to December 2018.

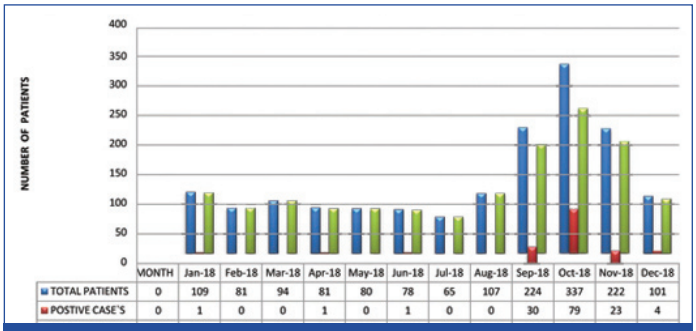
Out of the total patients tested in 2017, 22 (36%) patients were positive for NS1 antigen, 17 (28%) patients were positive for IgM antibody, 4 (7%) patients were positive for IgG antibody, 9 (15%) patients were positive for NS1 and IgM both, 4 (7%) patients were positive for IgM and IgG antibody, and 4 (7%) patients were positive for all three NS1, IgM, IgG [Table/Fig-4] .

In 2018, about 105 (76%) were positive for NS1 antigen out of total patients tested for dengue infection, 21 (15%) were positive case with IgM antibody, only 3 (2%) patients positive for IgG antibody,



MONTH	Jan-17	Feb-17	Mar-17	Apr-17	May-17	Jun-17	Jul-17	Aug-17	Sep-17	Oct-17	Nov-17	Dec-17
TOTAL PATIENTS	0	29	20	26	16	24	25	42	53	111	80	73	62
POSITIVE CASE'S	0	0	0	1	0	0	0	0	2	28	13	13	3
NEGATIVE CASE'S	0	29	20	25	16	24	25	42	51	83	67	60	59

[Table/Fig-2]: Showing total tested patients and positive and negative out of them (seasonal variation) in 2017.



MONTH	Jan-18	Feb-18	Mar-18	Apr-18	May-18	Jun-18	Jul-18	Aug-18	Sep-18	Oct-18	Nov-18	Dec-18
TOTAL PATIENTS	0	109	81	94	81	80	78	65	107	224	337	222	101
POSTIVE CASE'S	0	1	0	0	1	0	1	0	0	30	79	23	4

[Table/Fig-3]: Showing total patients tested and positive and negative out of them (Seasonal variation) in 2018.

Month	No. of positive cases	NS1	IgM	IgG	NS1 and IgM	NS1 and IgG	IgM and IgG	NS1, IgM and IgG
Jan 2017	0	-	-	-	-	-	-	-
Feb 2017	0	-	-	-	-	-	-	-
Mar 2017	1	-	1	-	-	-	-	-
Apr 2017	0	-	-	-	-	-	-	-
May 2017	0	-	-	-	-	-	-	-
Jun 2017	0	-	-	-	-	-	-	-
Jul 2017	0	-	-	-	-	-	-	-
Aug 2017	2	1	1	-	-	-	-	-
Sep 2017	28	7	10	1	6	-	3	1
Oct 2017	13	7	4	1	1	-	-	-
Nov 2017	13	7	1	1	1	-	-	3
Dec 2017	3	-	-	1	1	-	1	-

[Table/Fig-4]: Seropositivity of different serological markers dengue infection in 2017.

5 (4%) were positive for both NS1 and IgM, 2 (1%) positive case for NS1 and IgG, 3 (2%) patients positive for IgM and IgG antibody and no case positive for all three NS1, IgM, IgG in 2018 [Table/Fig-5].

Month	No. of positive cases	NS1	IgM	IgG	NS1 and IgM	NS1 and IgG	IgM and IgG	NS1, Igm and IgG
Jan 2018	1	-	1	-	-	-	-	-
Feb 2018	0	-	-	-	-	-	-	-
Mar 2018	0	-	-		-	-	-	-
Apr 2018	1	-	-	-	-	-	1	-
May 2018	0	-	-	-	-	-	-	-
Jun 2018	1	-	1	-	-	-	-	-
Jul 2018	0	-	-	-	-	-	-	-
Aug 2018	0	-	-	-	-	-	-	-
Sep 2018	30	14	10	1	2	1	2	-
Oct 2018	79	68	8	1	2	-	-	-
Nov 2018	23	21	-	-	1	1	-	-
Dec 2018	4	2	1	1	-	-	-	-

[Table/Fig-5]: Seropositivity of different serological markers dengue infection in 2018.

In the platelet count analysis, out of the total positive dengue patients 126 (63.31%) cases were found with less than one lac platelet count and 93 (4.79%) out of total negative dengue patients were there with less than one lac platelet count [Table/Fig-6].

Patients tested for dengue infection	Patients consists of <100000 platelets/cumm
Positive cases (n=199)	126 (63.31 %)
Negative cases (n=1941)	93 (4.79 %)
Total	219

[Table/Fig-6]: Platelet count compared with positive and negative dengue infected patients.

DISCUSSION

Seroprevalence of dengue infection: This study investigates the DENV infection with seasonal variation. In the present study, total 2140 samples were included in the study, out of which 199 (9.29%) cases were positive for one or more serological parameters of dengue infection. Out of the total 199 dengue seropositive cases, NS1 was positive in 127 (63.81%) cases, 38 (19.09%) cases were IgM positive, only 7 (3.51%) cases were positive for IgG, and 27 (13.56%) cases were positive for combination of any two seromarkers (antigen and antibody) or all of them. A study conducted by Biradar A in Al-ameen Medical College, Karnataka [15] shows similar results, NS1 positive in 46.55% cases and IgM was positive in 6.89% cases and positive cases for IgG antibody was 24.13% and 22.43% cases were positive for both antigen and antibody or both antibodies, or combination of serological parameters [15]. Immunoglobulin's begins to appear in 5-10 days of fever in primary infection and after about 4-5 days in secondary DENV infection [16].

Age and gender distribution: Most of the positive cases were male of age group under 15 years (33.16%). Out of the total positive cases in this study 136 (68.34%) males are infected and only 63 (31.65%) females are infected. Reduced disease detection in women may be due to low reporting and care-seeking for women and that determining sex differences requires well designed study [17]. Males were more infected then female which might be due to increased exposure at work places or outdoor activities. It had been suggested by Halstead SB et al., that immune responses in females are more competent than in males, resulting in greater production of cytokines rendering them more immune to dengue infection than males [18]. A study conducted by Pandey N et al., at Lucknow, North India (2008-2010), showed similar result as this study, 853 (54.43%) patients were under the age of 15 years [19], whereas in present study 92 (46.23%) patients were under 15 years of age out of total (199) positive dengue patients. So it can be said that pediatric age group is more prone to dengue infection.

Seasonal variation: If authors compare the same study (conducted by Pandey N, at Lucknow, North India (2008-2010) [19]) on the basis of seasonal variations it also shows similar results – most of the positive cases were starting from August to late October [19]. In this study predominant epidemic of dengue was also observed from August to November.

In Varanasi, rainy season starts around late June and last till October. Rain, temperature and relative humidity are reported as the major and important climatic factors, which could not or collective be responsible for an epidemic [20]. In the north India, the largest proportion of serologically positive cases has been recorded in the post-monsoon period [20]. In the study done in Bangladesh, the seasonal occurrence of positive cases had shown that post-monsoon period is the most affected period [21]. Studies have proposed that ecological and climatic factors influence the seasonal prevalence of the vector *Aedes aegypti* and DENV [22].

Thrombocytopenia: In the present study, out of the 2140 suspected dengue infection cases, thrombocytopenia (platelet count <100000/cumm as per WHO guideline for DHF) [9] was observed in 219 (10.23%) cases and out of this 219 patients, 57.53% cases were positive for dengue infection and other 42.47% cases were negative for dengue infection. The average platelet count of dengue positive patients was higher than that of dengue negative. A study conducted by Kulkarni RD et al., thrombocytopenia was seen in 68.8% of dengue positive patients and whereas Tathe SS et al., reported 81.72 % in his study [23,24].

Limitation(s)

The dengue day-1 test was for in vitro diagnostic use only. This test detects the presence of Dengue NS1 antigen and IgM and IgG antibodies to DENV in the specimen and should not be used as the sole criteria for the diagnosis of DENV infection.

CONCLUSION(S)

This study concluded that, dengue infection cases are more common in rainy and postrainy season in northern part of India. For the diagnostic purposes NS1 antigen is the best marker in early phase of infection. Apart from that IgM and IgG are the confirmatory indicators in later phase of infection.

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