

**ORIGINAL ARTICLE****To study the prevalence of dengue viral infection among clinically suspected patients**

Hitesh Punyani

Associate Professor, Department of General Medicine, Major S D Singh Medical College &amp; Hospital, Farrukhabad, Uttar Pradesh, India

**ABSTRACT:**

**Aim:** The present study aims to estimate the prevalence of dengue viral infection among patients who exhibit clinical symptoms and seek medical attention at a tertiary care centre. **Material and methods:** This was a prospective observational study conducted in the Department of General Medicine. A total of 560 serum samples from suspected dengue cases attending OPD or admitted in the hospital were tested for the confirmation of Dengue. We have received blood samples in our microbiology laboratory, the blood samples were allowed to clot at room temperature and then we centrifuged the samples and serum samples were separated. From the serum samples we have done NS1 Ag and IgM Ab testing by ELISA. **Results:** Out Of 560, 100 samples were positive for dengue. Seroprevalence of Dengue was 17.86%. Out of 100 dengue patients 64(64 %) were male patients and 36 (36%) were female patients. All dengue positive patients in our study had fever of 2 to 7 days. The most common presenting symptoms of dengue were fever with body ache (44%), headache (36%), nausea (32%) and vomiting (21%). Out of 100 dengue cases fever with rash was observed in 6 cases (6%). **Conclusion:** The present results revealed that the study region is epidemic for dengue viral infection and there is an urgent need for the constant monitoring to control further spreading of the infection in the community, hence serological test have important role in the early diagnosis. Therefore IgM ELISA is recommended in all the suspected dengue patients so as to instigate essential treatment and assessment of morbidity and mortality rate during an outbreak.

**Keywords:** IgM ELISA, Dengue viral infection, Aedes aegypti.

**Corresponding Author:** Hitesh Punyani, Associate Professor, Department of General Medicine, Major S D Singh Medical College & Hospital, Farrukhabad, Uttar Pradesh, India

**This article may be cited as:** Punyani H. To study the prevalence of dengue viral infection among clinically suspected patients. J Adv Med Dent Sci Res 2015;3(4):187-190.

**INTRODUCTION**

Dengue fever is presently regarded as the most significant viral disease transmitted by arthropods, owing to its extensive geographic range spanning over 100 nations and its capacity to cause severe and potentially fatal outbreaks of illness. Currently, a staggering 2.5 billion individuals, which constitutes 40% of the global populace, are susceptible to contracting dengue fever. Annually, an estimated 50 to 100 million cases of this disease are reported across the globe.<sup>1</sup>

The Dengue virus was initially discovered in India in 1945, and it is prevalent in both urban and semi-urban regions. India has experienced a resurgence of Dengue virus, resulting in the emergence of cases of Dengue fever/DHF in diverse regions of the country over the past four decades.<sup>2</sup> During epidemics of dengue, the attack rates among those who are susceptible range from 40% to 90%. It is estimated that approximately 500,000 cases of dengue hemorrhagic fever (DHF) require hospitalization annually, with a significant proportion of these cases affecting children.<sup>3</sup>

The Dengue virus is classified under the genus Flavivirus and the family Flaviviridae, and is transmitted through mosquitoes. The Aedes aegypti vector is a diurnal mosquito species that poses a significant public health concern due to its breeding

habits in both natural and artificial aquatic environments. Dengue infections are the result of infection by one of four serologically associated viruses, classified as DEN-1, DEN-2, DEN-3, and DEN-4. Infection by any of the serotypes of dengue virus typically results in a mild, self-limiting febrile illness known as classical dengue fever. However, in rare instances, severe and life-threatening conditions such as dengue haemorrhagic fever and dengue shock syndrome may develop.<sup>4</sup>

The onset of classical dengue fever typically occurs within 4 to 6 days following an infective mosquito bite. Symptoms include sudden fever, often biphasic in nature, severe headache, chills, generalized muscle and joint pains, and may be accompanied by a maculopapular rash. The clinical presentation may include leukopenia, relative lymphocytosis, thrombocytopenia, and potential manifestation of hemorrhagic symptoms.<sup>5</sup>

The gold standard methods for identifying dengue infection, namely viral isolation through cell culture and subsequent detection via immunofluorescence, are not readily available to peripheral and even most tertiary care laboratories.<sup>6</sup> The identification of dengue-specific IgM/IgG has been the primary diagnostic method for detecting dengue infection over an extended period. The indirect approach of antibody detection in diagnosis is susceptible to both erroneous

positive and negative outcomes.<sup>7</sup> The NS1 antigen can be detected from the onset of fever in both primary and secondary infections. The NS1 protein has been demonstrated to exhibit a high degree of specificity as a viral marker, rendering it a highly dependable parameter for the detection of dengue infection from the onset of fever.<sup>8</sup>

A subset of individuals who have encountered a particular dengue serotype in the past may exhibit hemorrhagic manifestations and endothelial permeability upon subsequent exposure to a distinct dengue serotype. The World Health Organization (WHO) reclassified the syndrome previously known as dengue hemorrhagic fever and dengue shock syndrome in 2009, now referring to it as severe dengue. The medical condition known as severe dengue has also been referred to as dengue vasculopathy. The occurrence of vascular leakage in these individuals can result in hemoconcentration and serous effusions, which may ultimately lead to circulatory collapse. The co-occurrence of severe hemorrhagic complications can result in a shock syndrome that presents a higher risk of fatality compared to bleeding alone.<sup>9</sup> The Indian sub-continent is known to have endemic cases of Dengue. Dengue has emerged as a significant public health concern in India, characterized by rapid urban epidemics.<sup>10</sup>

## RESULTS

**Table 1-Seroprevalence of Dengue**

Total no of patients	Dengue positive patients	%
560	100	17.86

Out Of 560, 100 samples were positive for dengue. Seroprevalence of Dengue was 17.86%. Table 1

**Table 2- Demographic profile of patients**

Gender	N=100	%
Male	64	64
Female	36	36
<b>Age years</b>		
Below 10	11	11
10-20	24	24
20-30	28	28
30-40	21	21
40-50	9	9
Above 50	7	7
<b>Area</b>		
Urban	68	68
Rural	32	32

Out of 100 dengue patients 64(64 %) were male patients and 36 (36%) were female patients. Out of 100 dengue patients, 68(68%) patients were from urban area and 32(32%) from rural area. In our study dengue infection was observed more (28%) in the age group 20 to 30 years followed by 10 to 20 years (24%) and 30 to 40 years (21%).

**Table 3-Clinical profile of dengue patients**

Clinical presentation	Number	%
Fever + myalgia	11	11
Fever + rash	6	6
Fever + headache	36	36

## MATERIAL AND METHODS

The study was conducted prospectively and observed in the Department of General Medicine, following approval from the protocol review committee and institutional ethics committee. A cohort of 560 serum samples obtained from individuals suspected of having contracted Dengue and either attending the Outpatient Department or being admitted to the hospital were subjected to diagnostic testing for the purpose of Dengue confirmation. This study included patients from all age groups. A patient exhibiting symptoms such as headache, retro-orbital pain, myalgia, arthralgia, rash, and haemorrhagic manifestation was suspected to have contracted dengue.

The study utilized serum samples obtained from the patients to conduct tests for Dengue NS1 antigen and dengue IgM antibody. The Dengue NS1 antigen capture ELISA and dengue IgM capture ELISA, both developed by PanBio Diagnostics, were employed to confirm the presence of dengue cases. The ELISA assays were conducted in accordance with the instructions provided by the manufacturer. Blood samples were received and allowed to clot at ambient temperature in the microbiology laboratory. The samples were then subjected to centrifugation, resulting in the separation of serum samples. ELISA was employed to conduct NS1 antigen and IgM antibody testing on the serum samples.

Fever+ nausea	32	32
Fever + vomiting	21	21
Fever + arthralgia	14	14
Fever + bodyache	44	44
Fever + itching	12	12

All dengue positive patients in our study had fever of 2 to 7 days. The most common presenting symptoms of dengue were fever with body ache (44%), headache (36%), nausea (32%) and vomiting (21%). Out of 100 dengue cases fever with rash was observed in 6 cases (6%).Table 3.

**Table 4-Serology results of rapid dengue tests**

Test results	No. of patients	%
NS1/NS1+IgM/IgM Positive	87	87
IgG Positive	8	8
IgG + IgM Positive	5	5
Total	100	100

Out of 100 dengue cases, NS1/NS1+IgM/IgM were positive for 87(87%) patients, suggesting primary infection. IgM and IgG positive was seen in 8(8%) patients, suggesting late primary or early secondary infection. IgG was positive in 5(5%) cases, suggesting secondary or past infection. Out of all dengue cases thrombocytopenia ( $<1,00,000/\text{mm}^3$ ) was observed in 35 cases. In 7 patients platelet count was  $<20,000/\text{mm}^3$

## DISCUSSION

Serological diagnosis of dengue virus infection using a commercial capture ELISA of both IgM and IgG distinguishes primary and secondary infections is preferred. ELISA is a simple, reliable and cost effective method in the diagnosis of dengue infection. Total 554 blood samples of the patients suspected of having dengue infection were tested in the laboratory by rapid immunochromatography tests for NS1 Ag, IgG and IgM. Out of these 100 samples were positive for dengue. Seroprevalence of Dengue was 17.86%. 11.92% prevalence was reported by Chakravarti A et al<sup>11</sup> 18.99% prevalence was observed by Chaturvedi UC et al in Rajasthan.<sup>12</sup> Low prevalence 3.55% was reported by Gupta E et al.<sup>13</sup> A study from central India reported 31.3% prevalence rate.<sup>14</sup>

Out of 100 dengue patients 64(64 %) were male patients and 36 (36 %) were female patients. Similar result was observed by Gupta E et al, in their study out of total positive dengue cases, 62.63% were males and 37.37% females.<sup>13</sup> Many studies have observed higher prevalence of dengue infection among males than females.<sup>11,12,15,16</sup> Vermunt J.K., et al reported 46.6% male & 53.4 female dengue patients.<sup>16</sup> Study by Kale A V et al reported 63.33% were males & 36.66% were females.<sup>15</sup>

In our study, out of 100 dengue patients, 68(68%) patients were from urban area and 32(32%) from rural area. similar results was by Vermunt J.K., et al. 109 cases (75%) were from rural area where as 25 cases (25%) were from urban area.<sup>16</sup> According to their report the rural broaden of dengue infection is comparatively a recent phenomenon which is supposed to be linked with the shortage of water in rural areas, designing of schemes for water supply to

the rural areas and development of newer water transport system in the rural places.

In our study dengue infection was observed more (28%) in the age group 20 to 30 years followed by 10 to 20 years (24%) and 30 to 40 years (21%). Gupta E et al in their study observed maximum dengue cases in age group 10-20 years (31.58%) and 21to30yrs. (15.78%).<sup>13</sup> Kale et al,observed commonest age group affected was (34%) was between 11-15 years.<sup>15</sup> Some Indian studies have reported that dengue infection is more common in children.<sup>16-18</sup>

All dengue positive patients in our study had fever of 2 to 7 days. The most common presenting symptoms of dengue were fever with body ache (44%), headache (36%), nausea (32%) and vomiting (21%). Out of 100 dengue cases fever with rash was observed in 6 cases (6%). Similar clinical presentation was observed by Gupta E et al, fever was present in almost all cases (n=380) followed by, headache (n=274), joint pain (n=2432), myalgia (n=144), retro-orbital pain (n=141), backache (n=95), skin rash (n=80).<sup>13</sup>

Out of 100 dengue cases, NS1/NS1+IgM/IgM were positive for 87(87%) patients, suggesting primary infection. IgM and IgG positive was seen in 8(8%) patients, suggesting late primary or early secondary infection. IgG was positive in 5(5%) cases, suggesting secondary or past infection. Gupta E et al reported that, Out of the 380 dengue positive cases, 136(35.79%) were NS-1 positive, 117(30.79%) were IgM positive, 38(10%) were IgG positive, 71(18.68%) were IgG/IgM positive, 14(3.68%) were IgG NS-1/IgMNS-1 positive and 4(1.05%) were IgGIgMNS-1 positive.<sup>13</sup>

Though among methods used for diagnosis of dengue the virus isolation, molecular methods are more specific tests, facilities are not available in all institutes. Serological tests are most commonly used in most of the laboratories. Dengue virus specific IgM antibodies tend to appear as early as 3 days after infection and remains in circulation for 30 to 60 days. IgG antibodies arise at about 7 days, they reach a peak at 2-3 weeks and persists for life long.<sup>18</sup> NS1 detection has been a promising test to diagnose dengue in its early febrile stage. The NS1 protein was found to be highly conserved in all dengue serotypes, circulating

in high levels during the first few days of illness. It correlates with the development of Dengue Fever. There is no cross reaction of the dengue NS1 protein with those of other related *flavi viruses*.<sup>19,20</sup> Out of all dengue cases thrombocytopenia (<1, 00,000/mm<sup>3</sup>) was observed in 35 cases. In 7 patients platelet count was < 20,000/mm<sup>3</sup>. One of the WHO diagnostic criteria for DHF is Thrombocytopenia: <1 lakh/mm<sup>3</sup>. Chakravarti A et al reported thrombocytopenia in 51.5% patient.<sup>11</sup> Kale A V et al observed thrombocytopenia in 56% patients, platelet count <40,000 in 33.33% cases.<sup>15</sup> Platelet count less than 1, 00,000/ml was noticed in 220 cases (68.75%), report published by R D Kulkarni et al.<sup>21</sup>

### CONCLUSION

The present results revealed that the study region is epidemic for dengue viral infection and there is an urgent need for the constant monitoring to control further spreading of the infection in the community, hence serological test have important role in the early diagnosis. Therefore IgM ELISA is recommended in all the suspected dengue patients so as to instigate essential treatment and assessment of morbidity and mortality rate during an outbreak.

### REFERENCE

1. Wichmann O, Hongsiriwon S, Bowonwatanuwong C, Chotivanich K, Sukthana Y, Pukrittayakamee S. Risk factors and clinical features associated with severe dengue infection in adults and children during the 2001 epidemic in Chonburi, Thailand. *Tropical Medicine and International Health*. 2004 Sep 1;9(9):1022-9.
2. Singh B. Dengue outbreak in 2006: Failure of public health system?. *Indian Journal of Community Medicine*. 2007 Apr 1;32(2):99.
3. Chaturvedi UC, R Shrivastava. Dengue Haemorrhagic fever: A global challenge. *IJMM*. 2004;22(1):5-6.
4. Yamashiro T, Disla M, Petit A, Taveras D, Castro-Bello M, Lora-Orste M, et al. Seroprevalence of IgG specific for dengue virus among adults and children in Santo Domingo, Dominican Republic. *The American journal of tropical medicine and hygiene*. 2004 Aug 1;71(2):138-43.
5. Topley WWC, Wilson SGS. *Topley and Wilson Microbiology and Microbial infections*, 10th Edition. 2006;993-1009.
6. Chakravarti A, Kumaria R, Batra VV, Varma V. Improved detection of dengue virus serotypes from serum samples – Evaluation of single-tube multiplex RT-PCR with cell culture. *Dengue Bulletin*. 2006;30:133-40.
7. Peeling RW, Artsob H, Pelegrino JL, Buchy P, Cardosa MJ, Devi S. Evaluation of diagnostic test: Dengue. *Nat Rev Microbiol*. 2010;8:S30–7.
8. Datta S, Wattal C. Dengue NS1 antigen detection: A useful tool in early diagnosis of dengue virus infection. *Indian J Med Microbiol*. 2010;28(2):107-10.
9. Statler J, Mammen M, Lyons A, Sun W. Sonographic findings of healthy volunteers infected with dengue virus. *J Clin Ultrasound*. 2008;36(7):413-7.
10. Dar L, Broor S, Sengupta S, Xess I, Seth P. The first major outbreak of dengue hemorrhagic fever in Delhi, India. *Emerg Infect Dis*. 1999;5(4):589-90
11. Chakravarti A, Arora R, Luxemburger C. Fifty years of dengue in India. *Trans R Soc Trop Med Hyg*. 2012;106(5):273-82
12. Chaturvedi UC, Nagar R. Dengue and dengue haemorrhagic fever: Indian perspective. *J Biosci*. 2008;33(4):429-441
13. Gupta E, Dar L, Kapoor G, Broor S. The changing epidemiology of dengue in Delhi, India. *Virology*. 2006;3:2003-7.
14. Ukey PM, Bondade SA, Paunipagar PV, Powar RM, Akulwar SL. Study of Seroprevalence of Dengue Fever in Central India. *Ind J Community Med*. 2010; 35(4): 517-19
15. Kale AV et.al, clinical profile and outcome of dengue fever from a tertiary care centre at Aurangabad Maharashtra India: an observational study, *IOSR journal of dental and medical sciences*, volume 13, issue 9 ver. Vii sep. 2012; 14-19
16. Vermunt J.K., Magidson J. Latent class cluster analysis. *Appl. Latent Class Anal*. 2002;11:60.
17. Garg A, Garg J, Rao YK, Upadhyay GC, Sakhuja S. Prevalence of dengue among clinically suspected febrile episodes at a teaching hospital in North India. *J Infect Dis and Immun*. 2011; 3(5):85-89.
18. Vijaykumar TS, Chandy S, Satish N, Abraham M, Abraham P, et al. Is dengue an emerging as a major public health problem? *Ind J Med Res*. 2005;121:100-07
19. Shrivastava A, Dash PK, Tripathi NK, Sahni AK, Gopalan N, Lakshmana Rao PV. Evaluation of a commercial Dengue NS1 enzyme-linked immunosorbent assay for early diagnosis of dengue infection. *Indian J Med Microbiol*, 2011; 29(1):51-5.
20. Datta S, Wattal C. Dengue NS-1 antigen detection: A useful tool in early diagnosis of dengue virus infection. *Indian J Med Microbio* 2010; 28:107- 190.
21. Kulkarni R D, Patil S S, Ajantha G S, Upadhya A K, Kalabhavi A S, Shubhada R M, Shetty P C, Jain P A. Association of platelet count and serological markers of dengue infection- importance of NS1 antigen. *Indian J Med Microbiol* 2011;29:359-62