Section: Microbiology

Original Research Article

A STUDY ON SEROPREVALENCE OF DENGUE FEVER IN A TERTIARY CARE HOSPITAL, NORTH KERALA

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Abstract

Background: Dengue virus infection which has emerged as a notable public health problem in recent decades is one of the most important human arboviral infections transmitted by the bite of infected Aedes mosquitoes. The aim is to study the trend of seroprevalence of dengue infection and to highlight the detection of NS1antigen and IgM antibody in early diagnosis of dengue. **Materials and Methods:** This cross-sectional study was done in the Department of Microbiology of a tertiary care centre during the study period of January to December in 2018. The serum samples were subjected to Dengue NS1 antigen, IgM / IgG antibody detection. **Result:** Out of 1194 samples tested, 258 samples (21.6 %) gave a positive serological test for acute dengue viral infection. Out of which, NS1 antigen was found to be reactive in 142(11.89%), IgM in 116(9.71%) samples. Both males and females are affected almost equally (male 21.8%, female 21.3%). The most affected age group was 20-29 years (34.4%) followed by 30-39 years (32.5%). Highest percentage of suspects and cases were seen during the months June-July (2.68%and 8.79%). **Conclusion:** There are no specific dengue treatment, early diagnosis is crucial to prevent complications like DHF and DSS. The NS1 Ag detection and IgM antibody detection plays an important role in diagnosis of acute dengue infection.

INTRODUCTION

Dengue is one of the most important arboviral infections in India. Dengue fever is caused by single positive stranded enveloped RNA virus of the flaviviridae family, genus flavivirus. Therre are four different serotypes of Dengue virus; DENV-1, DENV-2, DENV-3, and DENV-4, which are further sub classified based on their individual genotype.[1] By genetic sequence analysis, a fifth serotype (DENV-5) which follows sylvatic cycle has been detected in Sarawak state of Malaysia in October 2013.[2] DENV-1, DENV-2, DENV-3, and DENV-4serotypes have been isolated from India but DEN V1, DEN-2 are causing majority of infections. Dengue virus is transmitted by the bite offemale mosquitoes mainly of the species Aedes aegypti and, to a lesser extent, Aedes. albopictus. whichbites during the day time. It was estimated that 100 million cases of dengue fever, 500,000 cases of dengue haemorrhagic fever, and 25,000 deaths occured annually in 1998 making it the most important tropical infectious disease after malaria.[3] Perinatal transmission can occur, which may increase the symptomatic infection in the new-born. Dengue can also be spread through blood transfusion, needle stick injury or organ transplant.

Dengue virus infection is a notable public health problem in terms of the mortality and morbidity.[4] Dengue is endemic in many parts of India.[5] In India, the first epidemic of clinical dengue-like illness was recorded in Chennai in 1780 and the first virologically proved epidemic occurred in Kolkata and Eastern Coast of India in 1963- 1964.[6] Infant DHF/DSS was first reported in 1970.The case fatality of severe dengue in Asian countries is very high.

Dengue fever is a self-limiting illness of short duration known popularly as break-bone fever. The incubation period varies from 3 to 7 days after the bite from infected mosquito. Some infections remain asymptomatic, and the majority will develop classical dengue fever. Symptoms include high fever, headache, vomiting, retro-orbital pain, myalgia, arthralgia, rash, vomiting, muscle and joint pains, and bleeding manifestations. Recovery generally takes two to seven days. The illness may range from a mild, self-limited dengue fever to severe forms of dengue, dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS). DHF is frequently seen during a secondary dengue infection. However, in infants it may also occur during a primary infection due to

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maternally attained dengue antibodie.[7] After the onset of illness, the virus can be detected in serum, plasma, circulating blood cells and other tissues for 4–5 days. Infection by one serotype induces high titred neutralising antibody and recovery from infection provides lifelong immunity against reinfection by the same serotype but only partial protection against infection with the other serotypes. The major diagnostic methods are viral culture, viral RNA detection by RT-PCR and serological tests such as NS1 Ag detection, IgM and IgG capture ELISA. Virus isolation mainly used for research purposes can be done by inoculation into mosquito cell lines C6/36 and AP 61. RT-PCR which is more sensitive and specific, can be used for the detection of serotypes and viral load quantification. Dengue viral NS1 Ag is a highly conserved non-structural protein for all the serotypes which gets released from the dengue infected cells and appears early in the bloodstream. NS1 antigen stimulates strong humoral immune response. NS1 antigen is detectable from day 1 of fever and remains positive up to 18 days. Sensitivity of NS1 antigen –capture ELISA is depended on the level of viremia and host humoral immune response. NS1 Ag assay is a useful early diagnostic marker for dengue fever which allows rapid detection on the first day of fever, before antibodies appear. A positive NS1 test result confirms dengue virus infection without providing serotype information.

IgM appears first after 5 days of fever and disappears within 90 days 8and the antibody levels are consistent with acute-phase infection. Cross reactivity with several other flaviviruses like Zika virus, West Nile virus, and St. Louis encephalitis virus may lead to false positive results with IgM ELISA. MAC ELISA (IgM antibody capture ELISA) is the recommended serological test in India.

The dengue IgG detectable at low titre in 14-21 days and then slowly increases, and persist for months or years which often reactive with many flaviviruses. Of these three different tests, NS1 antigen detection had the highest sensitivity in the early stages while IgM detection was more sensitive in the later part of the illness. Positive dengue IgM and IgG tests for dengue antibodies in an initial blood sample means that the person became infected within recent weeks. According to WHO dengue-specific laboratory tests are often not required for acute management of cases but should be performed to confirm the diagnosis.

Dengue fever is usually a self-limited illness. Appropriate clinical management can save the lives of patients with DHF and DSS and mortality can be reduced to less than 1%.[9] Prevention of dengue virus transmission entirely depends on effective vector control measures or interruption of human–vector contact. Although there is no specific treatment available for dengue, accurate and timely diagnosis has an important role in individual case management and in planning and implementing control strategies.

AIM: The present study was undertaken to determine the seropositivity of NS1 antigen and IgM antibody among patients with acute febrile illness.

MATERIALS AND METHODS

Period of study: One year, January 2018 to December 2018

Ethical clearance: Ethical clearance and approval for the study protocol were attained from the Institutional Ethics Committees of Govt. Medical College, Kozhikode. Consent from individual patients was obtained prior to blood collection.

Inclusion Criteria

The patients with clinical feature suggestive of dengue fever and DSS/DHF cases irrespective of their age and sex were included in this study.

A total of 1194 serum samples (males-678, females-516) from suspected dengue fever cases were collected from patients with acute febrile illness, headache, myalgia, arthralgia, rashes and bleeding tendencies under aseptic precautions. Study group included both inpatients and outpatients belonging to age group 3 to 87 years.

A single blood sample (approximately 2-3 ml) was collected from clinically suspected patients by venepuncture according to the Clinical and Laboratory Standards Institute (CLSI) standard protocol and allowed to clot at room temperature. Samples were then centrifuged, serum separated, and subjected for NS1 antigen assay by QUALISA Dengue NS1 (QUALPRO DIAGNOSTICS), dengue-specific IgM antibodies by using IgM antibody capture enzyme-linked immunosorbent assay (NIV DEN IgM Capture ELISA kit from National institute of virology). The tests were done according to the manufacturers guidelines and results were recorded based on OD value. Few samples were tested for dengue IgG. Data were analysed.

Statistical Analysis

Data was entered into Microsoft Excel and analysed using PASW (Predictive Analytics Software) Version 18.0. Descriptive statistics such as percentages were used. Inferential statistics such as Chi- square test were used to find out association between variables. Differences with p- value <0.05 were considered statistically significant.

RESULTS

Out of 1194 samples tested, 258 samples (21.6 %) gave a positive serological test for acute dengue viral infection [Figure 1]. Out of 258 seropositive cases, NS1 antigen was found to be reactive in 116 (9.71%) and IgM in 142 (11.89%), samples [Table 1] Seropositivity more during the month of July, (25.88% for NSI antigen and 15.85% for IgM antibody) [Table 2]. Both males and females were affected almost equally (male 57.36%, female 46.64%) with a male to female ratio 1.3:1. [Table 3]

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The association between gender and dengue sero-positivity was not found to be statistically significant [Table 4]. Seasonal variation was observed. Only few cases were present during the month of January to May and positivity peak noted between the months of June to September [Figure 2&3]. The most affected age group was 20-29 years followed by 30-39 years as 34.4% and 32.5% [Figure 4]. The association between age and dengue sero-positivity was found to be statistically significant. It was found that dengue sero-positivity was found to be more among those aged 50years or younger as compared to those older than 50years. There is 2 times more risk of contacting dengue fever in those aged ≤ 50 years. [Table5]

It is noticed that infection was prevalent throughout the year with seasonal spurts in monsoon and post monsoon. Highest percentage of suspects and cases were seen during the months June-July (2.68%and 8.79%). Dengue sero-positivity was found to be more during Monsoon Season (27.2%) as compared to other seasons and the association was found to be statistically significant (p- value<0.001) [Table 6].

Table 1: Age wise distribution of cases

Age in years	Total cases	NS1	IgM
0-9	12	2	0(16.6%)
10-19	56	3	2((8.92%)
20-29	180	30	32(34.4%)
30-39	240	32	46(32.5%))
40-49	364	24	30 (14.83%)
50 and above	342	25	32 (16.7%)
Total	1194	116	142(21.6%)

Table 2: Month wise distribution of cases

Month	Total samples tested for Dengue NSI and IgM	Samples positive for NS1	Samples positive for Dengue IgM	Total NS1+IgM
January	24	3	1	4
February	33	0	3	3
March	25	0	2	2
April	13	1	2	3
May	12	4	6	10
June	57	19	13	32
July	255	66	39	105
August	84	13	20	33
September	357	19	16	35
October	133	7	11	18
November	117	6	3	9
December	84	4	0	4
Total No	1194	142	116	258

Table 3: Distribution of Dengue by gender

Gender	Total cases	NS1 positive	IgM positive	Percentage
Male	678	78	70	57.36%
Female	516	64	46	42.64%
Total cases	1194	142	116	100%

Table 4: Gender distribution and association with Dengue seropositivity

Gender	Dengue Positive	Negative	Total	p-Value	Odd's Ratio (95% CI)
MALE	148 (21.8)	530 (78.2)	678 (100)	0.88	1.03 (0.78-1.362)
FEMALE	110 (21.3)	406 (78.7)	516 (100)		
TOTAL	258 (21.6)	936 (78.4)	1194 (100)		

Table 5: Association between age and Dengue seropositivity

Age	Dengue Positive	Dengue Negative	Total	p-Value	Odd's Ratio (95% CI)
≤ 50YRS	201 (25.3)	594 (74.7)	795 (100)	0.00001863 (<0.001)	2.03 (1.47- 2.804)
> 50 YRS	57 (14.3)	342 (85.7)	399 (100)		
TOTAL	258 (21.6)	936 (78.4)	1194 (100)		

Table 6: Association of Dengue seropositivity and seasonality

Season	Dengue Positive	Dengue NEGATIVE	TOTAL	P-Value	Odd's Ratio (95% CI)
Monsoon Season	205(27.2)	548(72.8)	753 (100)	<0.0000001	2.74 (1.97-3.80)
Other Season	53(12.1)	388(87.9)	441 (100)		
Total	258 (21.6)	936 (78.4)	1194 (100)		

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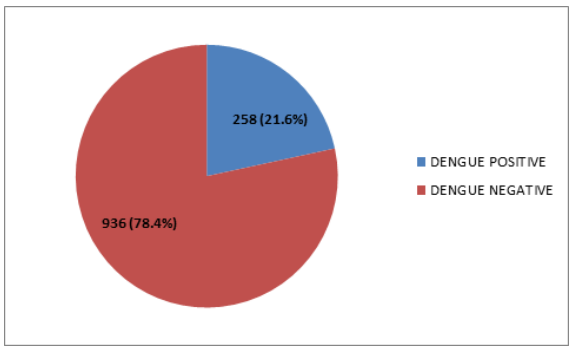

Figure 1: Positive cases of Dengue

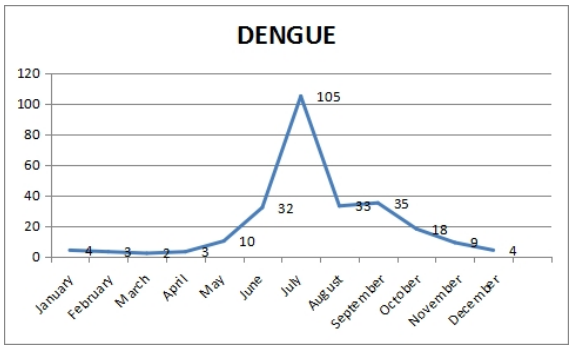

Figure 2: Seasonal distribution of Dengue cases

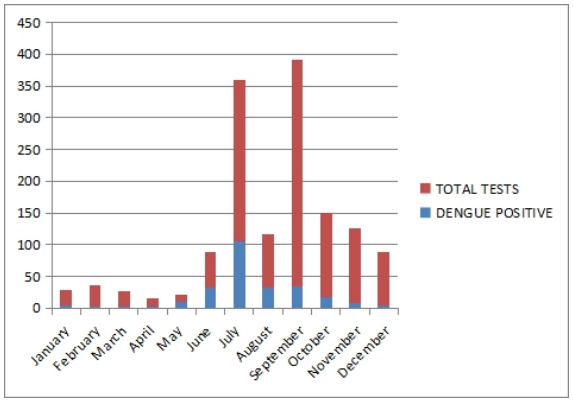

Figure 3: Seasonal distribution of Dengue positive cases out of total tested

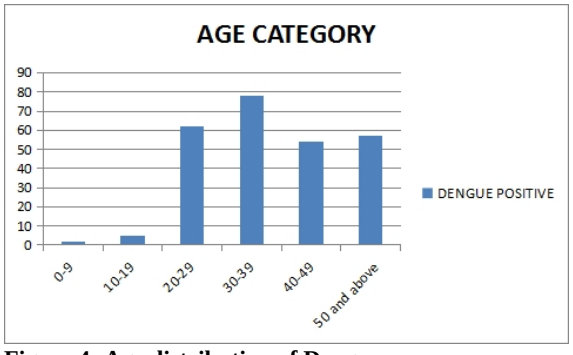

Figure 4: Age distribution of Dengue cases

DISCUSSION

Dengue virus is a mosquito-borne enveloped single-stranded RNA virus which belongs to Flaviviridae family, genus Flavivirus and is transmitted by mosquitoes especially Aedes egypti. Dengue fever is also known as break bone fever because of severe muscle, joint and bone pains. Dengue is a Spanish homonym for the "Swahili ki denga pepo" which describes a sudden, cramp like seizure caused by an evil spirit. Five serotypes of the virus have been found, all of which can cause disease. People of all age groups who are exposed to infected mosquitoes are victims of dengue fever.

According to World Health Organization (WHO), dengue fever is a mosquito-borne viral infection, found in tropical and sub-tropical climates worldwide, mostly in urban and semi-urban areas**.** Based on severity of infection there are two stages of dengue, dengue with or without warning signs and severe dengue (2009 WHO classification). The first recorded epidemic of dengue was reported in the late 18th century which affected Asia, Africa, and North America. Dengue haemorrhagic fever (DHF) a serious complication of dengue fever, and a life-threatening emergency is characterised by increased vascular permeability and abnormal blood clotting mechanisms. Dengue shock syndrome can occur after 2-7 days of dengue haemorrhagic fever.

Virus isolation, nucleic acid antigen or antibody detection can be used to diagnose the acute dengue infection. In acute phase of infection, serology is the method of choice for diagnosis. Of the NS1Ag, IgM and IgG tests used to diagnose dengue, NS1 antigen detection had the highest sensitivity in the early stages of illness while IgM detection was more sensitive in the later stage.

At present, there is no specific treatment available for dengue infection. Early diagnosis has a crucial role in management and implementation of control strategies. The first dengue vaccine, Dengvaxia (CYD-TDV) by Sanofi Pasteur, was first registered in Mexico in December, 2015 is available in some countries. Other methods of prevention include reducing mosquito habitat and limiting exposure to mosquito bites.

NS1 Ag and IgM detection by ELISA are potentially useful tests during acute dengue infection. In the present study, out of 1194 samples tested, 258 (21.6%) were serologically positive for NS1 Ag,142 (11.89%) and IgM antibody116 (9.71%) which suggests primary infection. Overall prevalence of dengue during the study period was 21.6%. In a study by Ashwini et al showed the highest positive detection rate of NS1 and IgM (97.8%).[10] In this study, a total of 94 cases were confirmed out of 112 clinically suspected cases with NS1 antigen positivity rate of 80.9 % and IgM 47.9%.

In the present study, out of 1194 suspected patients 116(9.71%) samples were IgM positive which indicated recent dengue infection. In a study by Sailaja et al, conducted in Andhra Pradesh, out of 108 cases, 33 (30%) were positive for IgM antibody between fifth to tenth day of fever.[11] In a study conducted by Neralwar A et al,[12] in Raipur, out of 1637 samples tested for Dengue infections, 161(29.92%) samples were NS1 positive, 83

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(15.42%) were both NS1 +IgM Ab positive and 294 (54.64%) were IgM Ab positive.

In this study out of 1194 samples 142 (11.89%) were positive for NS1 Ag. In a study by Fauziah MdKassim et al,[13] out of the 208 sera tested, 69.2% (144/208) sera were positive for dengue virus infection. Of these (32.2%) samples (67/208) were found positive for dengue NS1 antigen only. Datta et. al had reported NS1Ag positivity which varied from 71.42% to 28.4% in acute and early convalescent sera, conversely IgM detection rate was 93.61% and 6.38% in early convalescent and acute phase sere.[14]

In a study conducted in Angul district of Odisha by Dharitri Mahapatra et al out of the 1020 blood samples screened, 513 (50.2%) were positive for dengue NS1 Ag.[15]

The NS1 antigen and IgM antibody ELISA positivity rates are 25.59% and 17.3% respectively in study by Rajesh Kumar Varma et al conducted in Western Uttar Pradesh.[16] In another study conducted in Nepal, anti-dengue IgM was found in 8.5% of patients (50/590 cases).[17]

The IgG antibody level is very crucial in secondary infection of dengu**e** virus. In this study only 114 samples were tested for IgG dengue, of these 34 samples were positive.

To implement public health control programmes, it is nice to understand the male female difference in dengue infection rate and the complications of the disease. In this study higher prevalence of dengue infection was noted among males (57.36%) than females (42.64%) with a male: female ratio being1.3:1 seen in this study. The most affected age group was 20-29 years followed by 30-39 years as 34.4% and 32.5%. In another study by Hari.P Nepal et al the highest number of dengue cases was observed in the 21-30 years age group with greater predilection in males than in females. In this study 62% of positive cases were males (31/50) and 38% were females (19/50) with a, a male to female ratio of 1.6:1.[17]

In a study conducted in Rewa district of Madhya Pradesh, a total of 1113 sample tested for dengue the prevalence of dengue was higher in male (12.94%) in comparison to females (5.54%).[18] A male: female ratio being1.3:1. A male: female ratio of 2:1 seen in a study by Atul Garg et al.[19] In another study, females (57.5%) were more affected more than the males with a male to female ratio of 1:1.35.[19]

In this study the most affected age group was 20-29 years followed by 30-39 years as 34.4% and 32.5%. In a study by Manisha et al,[20] 21-30 years age group was most affected. In a study by Prabhakar et al,[5] the most affected age group was 15 to 30 years, with 13 (31.71%), followed by the 5--9-year age group, with 08 (19.51%) and the male-to female ratio was found to be 2.15:1.

The correlation between occurrence of dengue and monsoon season is clearly evident in this study and is further supported by similar findings from Delhi, Ludhiana, Lal et al,[21] 2007; and from Chandigarh by Ratho et al 2005.[22] In another study by Amitkumar et al, most of the samples 161 (82.14%) were received during monsoon and post monsoon period (June- November) with high positivity 27 (62.79%) for Dengue during post monsoon period (October-November).[23]

Prevention or reduction of dengue virus transmission depends mainly on control of the mosquito vectors or interruption of human-vector contact. Reduction of mosquito habitat to eliminate pockets of stagnant water that serve as mosquito breeding sites at home, workplaces and their vicinity, are the best way to prevent dengue fever stay in well-screened housing, protective clothing, usage of mosquito repellent may also reduce the dengue**.** Community participation, regular epidemiological surveillance, integrated vector control measures active participation of Government and non-government organizations for initiation of preventive strategies can reduce the rate of dengue.

CONCLUSION

According to the present study, dengue fever mainly seen in active adult male population. Increase in prevalence of dengue during monsoon and post monsoon season may be due to the abundant number of vectors and rapid urbanization. NS1 in combination with IgM assay offers the most sensitive and cost-effective diagnostic method for acute dengue infection.

REFERENCES

1. Clyde K, Kyle JL, Harris E. 2006, Recent advances in deciphering viral and host determinants of dengue virus replication and pathogenesis. J Virol; 80:11418-31.
2. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL et al 2013. The global distribution and burden of dengue. Nature. 496(7446):504–507.
3. Gubler DJ, 1998. "Dengue and dengue haemorrhagic fever," Clinical Microbiology Reviews, vol. 11, no. 3, pp. 480–496. View at Google Scholar · View at Scopus.
4. World Health Organization: Dengue haemorrhagic fever: Diagnosis, Treatment, Prevention and Control. 2nd ed. Geneva: World Health Organization; 1997. pp. 12–23. [Google Scholar]4.
5. Kaur H, Prabhakar H, Mathew P et al. 1997. Dengue haemorrhagic fever outbreak in October-November 1996 in Ludhiana, Panjab, India. Indian J Med Res.; 106: 1–3. [PubMed] [Google Scholar]
6. Gupta E, Dar L, Kapoor G, Broor S 2006. Prevalence of all the serotypes are found in India. The changing epidemiology of dengue in Delhi, India. Virol J. 3:92–96. [PMC free article] [PubMed] [Google Scholar].
7. Halstead SB, Lan NT, Myint TT et al. 2002; Dengue hemorrhagic fever in infants: Research opportunities ignored. Emerg Infect Dis. 8:1474–9. [PMC free article] [PubMed] [Google Scholar]
8. Sastry AS, Sandhya Bhat K, 2019; Essentials of Medical Microbiology; second edition;508-511.
9. Chaturvedi UC, Shrivastava R. 2004; Dengue haemorrhagic fever: A global challenge. Indian J Med Microbiol. 2004;22:5. [PubMed] [Google Scholar].
10. Ashwini Manoor, Anand,Sujatha Sistla, Rahul Dhodapkar, et al. 2016; Evaluation of NS1 Antigen Detection for Early Diagnosis of Dengue in a Tertiary Hospital in Southern IndiaClinDiagn Res. ; 10(4): DC01–DC04.

ISSN (O): 2687-5365; ISSN (P): 2753-6556

11. Sailaja BSG, Suneetha Rani M. 2019. Early detection of dengue by ELISA based method in a tertiary care hospital. MedPulse International Journal of Microbiology. August; 11(2): 18-21. https://www.medpulse.in/Microbiology.
12. Arvind Neralwar, BimalaBanjare, Barapatre R . 2015; Detection of NS1 antigen, IgM antibody for the diagnosis of dengue infection in patients with acute febrile illnessInt J Res Med Sci. Oct;3(10):2826-2830.
13. Fauziah MdKassim, M NurIzati, TAR TgRogayah, et al. 2011. Use of Dengue NS1 Antigen for early diagnosis of dengue virus infection. Southeast Asian J Trop Med Public HealthVol 42 No. 3 May 562-569.
14. Datta S, Wattal C. Dengue NS1 2010; Antigen infection An useful tool in early diagnosis of Dengue virus infection. Indian Journal of Medical Microbiology; 28(2):107–10. [PubMed] [Google Scholar].
15. Dharitri Mahapatra, Gitanjali Sarangi, Ashoka Mahapatra et al. 2014; Antigen Capture ELISA an Effective Method for Diagnosis of Early Dengue Infection - Report of an Outbreak at Angul District, Odisha, IndiaJournal of clinical and diagnostic research Volume : 8 | Issue : 8page DC08-DC10.
16. Rajesh Kumar Verma, Dharmendra Prasad Singh, SunitaKumari et al. 2016; Evaluation of ns1 antigen-capture elisa and igm antibody capture elisa in acute cases of dengue in rural population in a tertiary care teaching hospital in western Uttar Pradesh India International Journal of Pharmaceutical Sciences and Research IJPSR, Vol. 7(4): 1780-1784.
17. Hari P Nepal, Shamshul Ansari , Narayan Gyawali, et al. Detection of IgM against Dengue Virus in Clinically Suspected Patients Presenting at a TertiaryCare Centre, Narayani Zone, Nepal Journal of Tropical Diseases Volume 2 • Issue 3 • 1000139.
18. Taruna Singh, Amaresh Nigudgi, Vijay Tiwari, Pramod Kushwaha, Ashutosh Garg. 2022 .Emergence of Dengue as a Febrile Illness in Rewa and Nearby Districts of Madhya Pradesh During the Year, 2021: A Cross-sectional Study. Journal of clinical and diagnostic research Volume : 16 | Issue : 5 | Page : DC19 - DC23.
19. Garg A,Garg.J. Rao Y.K.Upadhaya GC, Shakuja.S 2011; Prevalence of dengue among clinically suspected febrile episodes at a teaching hospital in North India. Journal of infectious disease and immunity 3 (5):85-89.
20. Manisha P, Bhaumik P, Vicky G, Parvee S, Mahendra V. 2014; Seroprevalence of Dengue in Gujarat, Western India: A study at a tertiary care hospital. International J Med Science and Public Health; 3(1): 16-18.
21. Lal M, Aggarwal A, Oberoi M 2007. Dengue fever, an emerging viral problem in Ludhiana, North India. Ind. Jr. Community Med., 51: 198- 199.
22. Ratho RK, Mishra B, Kaur J, Kakkar N, Sharma K 2005. An outbreak of dengue fever in Peri Urban slums of Chandigarh, India, with special reference to entomological and climatic factors. Indian J. Med. Sci., 59: 519-27.
23. Amit Kumar, Rachana Kanaujia, Malay Bajpai 2020 Estimation of prevalence of dengue viral infection among clinically suspected patients attending a tertiary care centre in Uttar Pradesh, India International Journal of Advances in Medicine Kumar A et al. Int J Adv Med. Sep;7(9):1414-1417.

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