

Clinical Microbiology



DENGUE FEVER PREVALENCE IN RURAL AREA OF MATHURA DISTRICT OF UTTAR PRADESH.

Dr Prachee Singh*Assistant Professor, Department of Microbiology, K.D. Medical College and Hospital,
Mathura, Uttar-Pradesh. *Corresponding AuthorMs. Anju RaniAssistant Professor, Department of Microbiology, K.D. Medical College and Hospital,
Mathura, Uttar-Pradesh.

ABSTRACT Introduction-Among the 10 highest priority health issues presented, dengue was identified as one of the four main infections threatening global health by World Health Organization in 2019. In India, Dengue control comes under the purview of the NVBDCP, has been impeded by a lack of financial and human resources, poor availability of point-of-care diagnostics, and ineffective mosquito control methods. The dengue virus (DENV) is an RNA virus belonging to the genus flavivirus, and there are four serologically and genetically distinct serotypes, DENV 1–4. Infection can occur with any of the four serotypes, and multiple sequential infections can also occur. **Material &Methods-** This study was a hospital-based cross-sectional study conducted in the Microbiology Laboratory of Tertiary Care Hospital . The detection of positive cases was done by estimation of nonstructural protein 1 (NS1) antigen and immunoglobulin (Ig) M or IgG antibody. **Results-** 31.8% samples were found positive for dengue fever. Female patients have registered more (35.29%) positive share than that of male (29.20%) patients. Highest number of positive cases (45.07%) were registered in 19-30years age bracket followed by 37.5% in age bracket of more than 60 years age . The lowest (16.32%) positive cases have more than 200000 µl platelets. IPD patients had more (38.32%) positive cases than OPD patients (16.67%). **Conclusion-** In the present study, only the primary infection of Dengue fever was detected . There was no case found suffering with secondary infection . Dengue hemorrhagic fever (DHF) has not been noticed in any case. The lowest positive cases in IPD patients than OPD patients.

KEYWORDS: Dengue, dengue hemorrhagic fever, Morbidity, Mortality, Febrile diseases. NCVBDC.

BACKGROUND

Dengue, a mosquito-borne disease is continuously increasing over the last few years. It has become a major public health concern in India. As per NCVBDC (National Center for Vector Borne Diseases Control) data 233251 of dengue were reported in 2022 alone. The number of cases 1.88 lacs approx. increased from 2020 to 2022 in India. The data for increase rate of cases every year creates concern for damage to human life. Surveillance and control on mosquito breeding appears to be the methods to minimize the damage to human life. With this concern in mind, the present study is being carried out.

Objectives Of Study

The present study will focus on the outcome of following objectives-

- a- Prevalence of Dengue cases in rural area of Mathura district of Uttar Pradesh.
- b- Gender wise impact of virus.
- c- Comparative study on infection of virus in children and adults.
- d- Age wise rate of infection.
- e- Impact of virus in terms of platelets count in Dengue positive cases.
- f- Impact of virus in terms of IPD v/s OPD treatment of positive cases.

Inclusion Criteria

The blood samples were collected from the patients having fever, nausea, vomiting, rash, myalgias, arthralgias and having leukopenia. The patients with complaint of Abdominal pain, persistent vomiting, clinical fluid accumulation such as ascites or pleural effusion, mucosal bleeding, lethargy, liver enlargement greater than 2 cm, increase in hematocrit, and thrombocytopenia were also included. The patients with hemorrhage and narrowed blood pressure were included on priority. The febrile patients having excluded from malaria and enteric fever are included in the study.

Exclusion Criteria

Non febrile patients with no other complaints listed above in inclusion criteria. Confirmed diagnosed cases for other ailments were also excluded from the study.

The present study has been carried out on the patients either examined as OPD patients or admitted in different wards of the hospital with febrile history. The samples were collected and brought to Microbiology lab with prior consent of patients of the hospital and tested.

Study Period -1^{st} Sept. 2023 to 30^{th} NOV. 2023 (Three months).

Sample Size – 239

Analysis of Data

Chi-Square test and Logistic Regression analysis is being used to analyze the data.

INTRODUCTION

Dengue is a mosquito-transmitted virus and the leading cause of arthropod-borne viral disease in the world. It is also known as breakbone fever due to the severity of muscle spasms and joint pain. dandy fever, or seven-day fever because of the usual duration of symptoms. Although most cases are asymptomatic, severe illness and death may occur. Aedes mosquitoes transmit the virus and are common in tropical and subtropical parts of the world. The incidence of dengue has increased dramatically over the past few decades, and the infection is now endemic in some parts of the world. A few people who were previously infected with one subspecies of the dengue virus develop severe capillary permeability and bleeding after being infected with another subspecies of the virus. This illness is known as dengue hemorrhagic fever. This activity reviews the etiology, presentation, evaluation, and management of dengue fever and examines the role of the inter professional team in evaluating, diagnosing, and managing the condition.^{1,2,3,4} Dengue fever is caused by any of four distinct serotypes (DENV 1-4) of single-stranded RNA viruses of the genus Flavivirus. Infection by one serotype results in lifelong immunity to that serotype but not to others.⁵⁶⁷. The typical incubation period for the disease is 4 to 7 days, but it can last from 3 to 10 days. Symptoms more than two weeks after exposure are unlikely to be due to dengue fever. Viremia may be present for 24 to 48 hours before the onset of symptoms. A complex interaction of host and viral factors then occurs and determines whether the infection will be asymptomatic, typical, or severe. Severe dengue fever with increased microvascular permeability and shock syndrome is thought to be associated with infection due to a second dengue virus serotype and the patient's immune response. However, cases of severe dengue do occur in the setting of infection by only a single serotype. Worsening microvascular permeability often transpires even as viral titers fall.

94198 cases have been reported in india upto 17th Sept.2023 (approx 6 months period) over year 2022 cases of 233251. In Uttar pradesh 5742 Cases reported during the same period over previous year 19821 cases. Highest 9770 cases have been reported from Kerala in 6 months period of 2023. Meghalaya state showed lowest 59 cases reported in 6 months.¹⁰

5

MATERIALAND METHODS

INDIAN JOURNAL OF APPLIED RESEARCH

This study was a hospital-based cross-sectional study conducted in the Microbiology Laboratory of Tertiary Care Hospital in rural area of Mathura district of Uttar pradesh, India, between September 2023 to Nov. 2023 . Institutional Ethical Committee permission was obtained. All patients with clinical suspicion of dengue-like illness (DLI), attending outpatient department (OPD) or inpatient department (IPD), were included in the study after obtaining their written consent. A blood sample was collected, and the Dengue Duo rapid card test was conducted for the detection of nonstructural protein 1 (NS1) antigen and immunoglobulin (Ig) M or IgG antibody estimation. All positive samples were tested for IgM enzyme-linked immunosorbent assay (ELISA) test using MAC-ELISA. Serological detection of viral antigens (such as NS1) or particular antibodies are the preferred microbiological assays.¹¹

Laboratory confirmation of the diagnosis can be done either directly by detecting viral components in the blood or indirectly by serological measures. The choice of test depends on the timeline of the clinical presentation. During the early illness of the febrile phase, detection of viral components in the circulation is highly sensitive. Viral nucleic acid in serum can be detected by means of reverse transcriptase polymerase chain reaction (RT-PCR) assay or by detection of the virus-expressed soluble non-structural protein 1 (NS1) by means of enzyme-linked immunosorbent assay (ELISA).¹² Serology to detect IgM and IgG has a place from the fifth day of illness and also helps in deciding between primary or secondary dengue infection. A high titre of haemagglutinin antibodies suggests secondary dengue infection.^{13,14}

Antibody tests are commonly used to detect dengue in patients who have been infected for more than 5 days. These tests detect antibodies that the body produces against the dengue virus. IgM and IgG are the two types of antibodies that these tests can identify. IgM antibodies are produced early in the infection, while IgG antibodies are produced later on.

Antigen tests are used to detect the presence of the dengue virus in a patient's blood. These tests are most accurate when performed within the first five days of symptoms, as the virus can only be detected during this period. Antigen tests are useful in identifying the specific serotype of the virus, which can help with disease surveillance and outbreak response.

Timeline of dengue biomarker appearance in patients experiencing primary and secondary infection . In primary infection (top panel), both nonstructural protein 1 (NS1) and virus can be detected from the onset of disease, with immunoglobulin M (IgM) appearing around day 3 of illness and immunoglobulin G (IgG) appearing toward the end of the acute period. Secondary infections (bottom panel) are characterized by the presence of IgG early in the acute phase of disease and a shorter duration of NS1 and virus detection. Note onset of severe (DMS]), primarily in secondary infections and at a time when virus and NS1 levels are falling.¹⁵

It is recommended to do the Dengue Fever NS1 Antigen test in the first 5 days of fever. After 7-10 days of continuous fever, the recommended test is Dengue fever antibodies IgG & IgM.¹⁶

Dengue hemorrhagic fever (DHF) can go to a higher grade of fever. It might also include variable bleeding manifestations, like bleeding from the nose, vomiting blood, passing blood in the stool; breathing difficulty; and cold clammy skin, especially in the extremities. During the second phase, the virus may attack blood vessels, causing capillaries to leak fluid into the space around the lungs (pleural effusion) or the abdominal cavity (ascites).

Dengue shock syndrome (DSS) is a severe complication of dengue fever that is caused when the body's immune system overreacts to the dengue virus. It can lead to a sudden drop in blood pressure and dehydration, and if not managed timely, it may lead to multiple organ failures.

A-Interpreting Dengue Fever Antigen NS1 Results

Index	Result	Remarks		
< 0.9	Negative	No detectable dengue NS1 antigen. The result does not rule out dengue infection		
		An additional sample should be tested for IgG & IgM serology in 7-14 days.		
6	INDIAN J	OURNAL OF APPLIED RESEARCH		

0.9 to 1.1	Equivocal	Repeat sample after 1 week			
>1.1	Positive	Presence of detectable dengue NS1			
		antigen. Dengue IgG & IgM serology			
		assays should be performed on follow up			
		samples after 5-7 days of onset of fever,			
		to confirm dengue infection.			
Note-		Recommended to do NS1 Antigen by			
		ELISA in the first 5 days of fever. After 7-			
		10 days of continuous fever, the			
		recommended test is Dengue fever			
		antibodies IgG & IgM by ELISA			
B-ANTIBO	DY DETECT	FED	REMARKS		
IgM	DIDLIDO		Primary Dengue		
IgM & IgG			I ata Primary / Farly Secondary		
igivi & Igo			Dengue		
IøG			Secondary / Past Dengue		

Specimen Types

Serum

Interpretation Of Results

- 1- A positive NS1 test result confirms dengue virus infection without providing serotype information.
- 2- A negative NS1 test result does not rule out infection. People with negative NS1 results have been tested for the presence of dengue IgM antibodies to determine possible recent dengue exposure.¹⁷

Detection of the dengue virus nonstructural protein 1 (NS1) antigen is suggestive of recent exposure and/or acute infection with dengue virus. This test should be used for diagnostic purposes only. Dengue NS1 antigenemia overlaps with dengue virus viremia and can be used as an acute phase marker for infection.

RESULTS

Blood samples have been collected from febrile patients with symptoms clinically similar to Dengue .Dengue IgM/IgG serological test and or NS1. tests being carried out used for testing sera using standard method.

Table 1- Distribution Of Positive Cases As Per Serological Test-

Total	Positive	% of	Positive	Positive	% of	Total	Positive
sample	sample	positive	sample	sample	IgM	positive	sample
tested	with	sample	with	with	positive	sample	(%)
(n)	NS1	with	IgG	IgM	samples	(n1+n2)	
	(n1)	NS1	(n2)	(n3)	-	+n3)	
239	53	22.18%	Nil	23	9.62%	76	31.8%

Table 2- Gender Wise Distribution Of Positive Cases -

Gender	No. of total sample	Positive sample	% positive
	tested	(no)	sample
Male	137	40	29.20%
Female	102	36	35.29%
Total	239	76	31.8%

Table 3- Distribution Of Cases According To Age Groups

Age bracket	Total sample	Positive sample	% positive
(years)	tested (no)	(no.)	cases
0-18	49	08	16.32%
19-30	71	32	45.07%
31-45	43	13	30.23%
46-60	36	08	22.22%
>60	40	15	37.5%
Total	239	76	31.80%

Table 4- Distribution Of The Cases According To Platelet Count

Platelet count/µl of	No. of	Dengue positive	% of Dengue cases
blood	cases	(no.)	out of total no of
			positive cases
< 25000	06	02	2.63%
25000- 50000	10	05	6.57%
50000- 100000	27	18	23.68%
100000-200000	84	22	28.95%
>200000	112	29	38.15%
Total	239	76	

Table 5- Dengue Positive Ratio In IPD & OPD Patients-

Total IPD	Positive	Percent	Total OPD	Positive	Percent
Cases (n)	cases (n1)	positive(%)	cases (N)	cases(N1)	positive(%)
167	64	38.32%	72	12	16.67%

DISCUSSION

Out of 239 total samples tested in Microbiology Lab during three months period 76 (31.8%) samples were found positive Dengue fever cases (Table 1). In one such such study in western Rajasthan in early 2021 ,NS1 antigen was detected 62.80% followed by IgG 33.60%, 30.80% were IgM positive.¹⁸In other study the number of suspected patients tested for dengue and positivity by months during 2014-2017. The mean monthly dengue positivity during 2014-2017 ranged from 7.7% to $37.0\%^{19}$ In our study 22.18% samples are NS1 positive, IgG is nil and IgM positive cases are 9.62% samples. This difference may be because of change in demography and period of study. Female patients have registered more (35.29%) positive share than that of male (29.20%) patients (Table 2). In one other study carried out in western Uttar Pradesh, The proportion of male was higher than female among all seropositive cases, with the ratio of (M:F) 1.54:1 (P < 0.0001). The males were predominately more affected compared to females among rural (P=0.001) and semi-urban (P=0.016) areas. The positive NS1 (P = 0.004) and IgM (P = 0.0001) both results were significantly associated with male gender.²⁰ Highest number of positive case (45.07%) were registered in 19-30years age bracket followed by 37.5% positive cases in age bracket of more than 60 years age . The lowest (16.32%) positive cases were registered in children up to the age of 18 years (Table 3). In another study carried out in by Kerala Govt. in 2023, The overall seroprevalence in the 9-12 year age group is found low to moderate .There are regional variations; high burden and low burden clusters co-exist in the same districts. The actual burden of dengue exceeds the reported numbers. Heterogeneity in prevalence, the high proportion of inapparent dengue and the hyperendemic situation suggest the need for region-specific and targeted interventions, including vaccination.²¹ The platelets count below 25000 were recorded in 2.63% of positive Dengue cases. 38.15% of positive cases have more than 200000 platlets(Table 4).in one other sudy 16% of the patients had platelet count <20,000/µl among which 41% of the patients required platelet transfusion. The correlation between lower platelet count and transfusion requirement was found to be statistically significant (P < 0.001).²² IPD patients had more (38.32%) positive cases than OPD patients which was found 16.67% positive patients (Table 5). As per other study carried out OPD patients were more with 64.80% than. IPD (34.40%) and emergency (0.8%).²

CONCLUSION

In the present study, only primary infection of Dengue fever was detected. There was no case found suffering with secondary infection . Dengue hemorrhagic fever (DHF) has not been noticed in any case during the study period. The lowest positive cases were recorded in children. The positive cases have slightly more share in female patients than male . The present study observed more positive cases in IPD patients than OPD patients.

Financial Support and Sponsorship-Nil Conflicts of Interest- No conflict of interest

REFERENCES

- Schaefer TJ, Panda PK, Wolford RW. Dengue Fever. [Updated 2022 Nov 14]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK430732/ Baak-Baak CM, Cigarroa-Toledo N, Pech-May A, Cruz-Escalona GA, Cetina-Trejo
- 2. RC, Tzuc-Dzul JC, Talavera-Aguilar LG, Flores-Ruiz S, Machain-Williams C, Torres-Chable OM, Blitvich BJ, Mendez-Galvan J, Garcia-Rejon JE. Entomological and
- Chable OW, Britven BJ, Mendez-Galvan J, Garcia-Rejol JE. Entonloiogical and virological surveillance of dengue virus in churches in Merida, Mexico. Rev Inst Med Trop Sao Paulo. 2019 Feb 14;61:e9. [PMC free article] [PubMed] Sharma M, Glasner DR, Watkins H, Puerta-Guardo H, Kassa Y, Egan MA, Dean H, Harris E. Magnitude and Functionality of the NS1-Specific Antibody Response Elicited by a Live-Attenuated Tetravalent Dengue Vaccine Candidate. J Infect Dis. 2020 Mar 02021(6):457–872 (DMC Generatical) Durble Medl 3. 02;221(6):867-877. [PMC free article] [PubMed]. Oliveira LNDS, Itria A, Lima EC. Cost of illness and program of dengue: A systematic
- 4.
- Onverta ENDS, tutta A, Enna EC. Cost of miless and program of dengue: A systematic review. PLoS One. 2019;14(2):e0211401. [PMC free article] [PubMed] Seixas G, Salgueiro P, Bronzato-Badial A, Gonçalves Y, Reyes-Lugo M, Gordicho V, Ribolla P, Viveiros B, Silva AC, Pinto J, Sousa CA. Origin and expansion of the mosquito Aedes aegypti in Madeira Island (Portugal). Sci Rep. 2019 Feb 19;9(1):2241. [PMC free article] [PubMed] Chemi MA. Charlieri B. Use AK. Chen UK, Emmanuel O, Alcha Alibala LS. 5.
- Ghani NA, Shohaimi S, Hee AK, Chee HY, Emmanuel O, Alaba Ajibola LS. Comparison of Knowledge, Attitude, and Practice among Communities Living in 6.
- Comparison of Knowledge, Attitude, and Practice among Communities Living in Hotspot and Non-Hotspot Areas of Dengue in Selangor, Malaysia. Trop Med Infect Dis. 2019 Feb 15;4(1) [PMC free article] [PubMed] Maia LMS, Bezerra MCF, Costa MCS, Souza EM, Oliveira MEB, Ribeiro ALM, Miyazaki RD, Slhessarenko RD. Natural vertical infection by dengue virus serotype 4, Zika virus and Mayaro virus in Aedes (Stegomyia) aegypti and Aedes (Stegomyia) albopictus. Med Vet Entomol. 2019 Sep;33(3):437-442. [PubMed] Schaefer TJ, Panda PK, Wolford RW. Dengue Fever. [Updated 2022 Nov 14]. In: StraPaced [Uptamed] Texeura Icland (EL): SterDenced Publichizer 2003 Ion Available 7
- 8. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan. Available from: https://www.ncbi.nlm.nih.gov/books/NBK430732/ Murugesan A, Manoharan M, Dengue Virus. Emerging and Reemerging Viral Pathogens. 2020;281–359. doi: 10.1016/B978-0-12-819400-3.00016-8. Epub 2019 Sep
- 9 20. PMCID: PMC7149978
- https://ncvbdc.mohfw.gov.in/index4.php?lang=1&level=0&linkid=431&lid=3715 10

- Guzman MG, Halstead SB, Artsob H, Buchy P, Farrar J, Gubler DJ, et al. Dengue: A continuing global threat. Nat Rev Microbiol. 2010;8(Suppl):S7–16. [PMC free article] 11 [PubMed][Google Scholar] Peeling RW, Artsob H, Pelegrino JL, et al., Evaluation of diagnostic tests: dengue. Nat
- 12. Rev Microbiol 2010;8:Suppl:S30-8. [PubMed] [Google Scholar] Gubler DJ. Dengue and dengue hemorrhagic fever. Clin Microbiol Rev 13.
- 1998;11:480–96. [PMC free article] [PubMed] [Google Scholar] World Health Organization Regional office for South East Asia. Comprehensive guidelines for prevention and control of dengue and dengue haemorrhagic fever, revised 14
- and expanded edition. New Delhi: World Health Organization South East Asia regional office, 2011. [Google Scholar]
- David A. Muller, Alexandra C. I. Depelsenaire, Paul R. Young, Clinical and Laboratory Diagnosis of Dengue Virus Infection, The Journal of Infectious Diseases. Volume 215.
- Justices of Derige virus intervention, the round of intervention Diseases, while 21.5, Issue suppl_2, 1 March 2017, Pages S89–S95, https://doi.org/10.1093/infdis/jiw649 Wang SM, Sekaran SD. Early diagnosis of Dengue infection using a commercial Dengue Duo rapid test kit for the detection of NS1, IGM, and IGG. Am J Trop Med Hyg. 16 2010 Sep;83(3):690-5. doi: 10.4269/ajtmh.2010.10-0117. PMID: 20810840; PMCID: PMC2929071.
- Dengue internet CDC https://www.cdc.gov/dengue/index.html
- Prakash P, Gupta E, Nareda P, Gupta E, Agarwal R, Ashopa V, Beelwal A. Seroprevalence and incidence of primary dengue infection and its correlation with fetomaternal prognosis in Western Rajasthan. J Family Med Prim Care. 2023 Aug;12(8):1525-1530. doi:10.4103/jfmpc.jfmpc_1176_22. Epub 2023 Aug 29. PMID: 2776724): MCUP: PMCUP. 2010;521875 37767431; PMCID: PMC10521835.
- ST/6/451, FMCID: FMC10521555.
 Manoj Murhekara, Vasna Joshua a, K. Kanagasabaia, Vishal Shete a, M. Ravia, R. Ramachandrana, R. Sabarinathana, B. Kirubakarana, Nivedita Guptab Sanjay Mehendale, Epidemiology of dengue fever in India, based on laboratory surveillance data, 2014–2017; International Journal of Infectious Diseases 845 (2019) S10–S14 19
- Pillaveetil Sathyadas Indu, Thekkumkara Surendran Anish, Sujatha Chintha, Gnanaseelan Kanakamma Libu, Lawrence Tony, Etal; The burden of dengue and force 20 Government of Kerala-WHO Dengue study; The Lancet Regional Health – Southeast Asia 2023;*: 100337 Published Online XXX https://doi.org/10.1016/j.lansea. 2023.100337
- Manoj Kumar, Rajesh Kumar Verma, and Bishal Mishra ; Prevalence of Dengue Fever in Western Uttar Pradesh, India: A Gender-Based Study; Int J Appl Basic Med Res. 2020 Jan-Mar; 10(1): 8–11. Published online 2020 Jan 3. doi: 10.4103/ijabmr.IJABMR 337 18
- Asha J, Baiju NM, Innah SJ, Rafi A, John BM, Comparison of platelet indices in dengue 22. fever patients based on platelet transfusion: A prospective observational study in a tertiary care center. Asian J Transfus Sci. 2023 Jan-Jun;17(1):21-27. doi: 10.4103/ajts.AJTS_24_20. Epub 2022 Dec 12. PMID: 37188027; PMCID: PMC10180782
- Rakash, Prabhu & Gupta, Ekta & Nareda, Pooja & Gupta, Eshank & Agarwal, Richa & Ashopa, Vishakha & Beelwal, Anjali. (2023). Seroprevalence and incidence of primary dengue infection and its correlation with fetomaternal prognosis in Western Rajasthan. Journal of Family Medicine and Primary Care. 12. 1525-1530. 10.4103/jfmpc.jfmpc_ 1176 22.