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Study of seroprevalence of dengue infection in clinically suspected cases of dengue

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Abstract
Dengue virus infection has emerged as a notable public health problem in recent decades in terms of the mortality and morbidity associated with it. Dengue is endemic in many parts of India and epidemics are frequently reported from various parts of India and abroad. The case fatality rate in patients with dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) can be as high as 44%. Hence based on above findings the present study was planned for Study of Seroprevalence of Dengue Infection in Clinically Suspected Cases of Dengue at NMCH, Patna.
The present study was planned in Department of Microbiology, Nalanda Medical College and Hospital Patna, Bihar, India. The study was conducted from August 2019 to December 2019. In the present study 909 patients of suspected dengue cases were evaluated. A single blood sample approximately 2-3 ml was collected from each patient in a plain vaccutainer tube with all aseptic precautions. Serum was subjected toNS1, IgG and IgM antibodies by enzyme linked immunosorbent assay (ELISA) Kit provided by NIV Pune.
The data generated from the present study concludes that Dengue cases were more during September to December in the post monsoon season which is useful to plan special preventive strategies. The study draws attention toward the male, young adult age group. Discrimination of primary and secondary dengue infection is important as the possibility of DHF and DSS are more in secondary infection.

Keywords: ELISA, dengue viral infection, aedes aegypti, etc

Introduction
Dengue is the most common and important arthropod-borne viral (arboviral) illness in humans. It is transmitted by mosquitoes of the genus Aedes, which are widely distributed in subtropical and tropical areas of the world. The incidence of dengue has increased dramatically in recent decades, with estimates of 40%-50% of the world's population at risk for the disease in tropical, subtropical, and, most recently, more temperate areas [1].
A small percentage of persons who have previously been infected by one dengue serotype develop bleeding and endothelial leak upon infection with another dengue serotype. This syndrome is termed severe dengue also known as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS).
Dengue fever is typically a self-limited disease with a mortality rate of less than 1% when detected early and with access to proper medical care. When treated, severe dengue has a mortality rate of 2%-5%, but, when left untreated, the mortality rate is as high as 20%.
Many individuals with dengue may be asymptomatic. Many patients with dengue experience a prodrome of chills; rash, including erythematous mottling of the skin; and facial flushing, which may last 2-3 days. Children younger than 15 years who have dengue usually have a nonspecific febrile syndrome, which may be accompanied by a maculopapular rash. Dengue should be suspected in individuals who present with high fever (104°F/40°C), retro-orbital headache, muscle and joint pain, nausea, lymphadenopathy, vomiting, and rash and who have travelled within 2 weeks of symptom onset to an area where appropriate vectors are present and dengue transmission may be occurring.
Dengue is the most common and important arthropod-borne viral (arboviral) illness in humans. Globally, 2.5-3 billion individuals live in approximately 112 countries that experience dengue transmission. While the annual incidence is unclear owing to incomplete global reporting and misclassification of illness, approximately 3.2 million individuals were infected globally in 2015. It is caused by infection with 1 of the 4 serotypes of dengue virus, which is a Flavivirus (a genus of single-stranded non segmented RNA viruses). Infection with one dengue serotype confers lifelong homotypic immunity to that serotype and a brief period (approximately 2 years) of partial heterotypic immunity to other serotypes, but an individual can eventually be infected by all 4 serotypes. Several serotypes can be in circulation during an epidemic.
Dengue is transmitted by mosquitoes of the genus Aedes, which are widely distributed in subtropical and tropical areas of the world. An individual with dengue is capable of transmitting the virus for 4-5 days (maximum, 12 days) to a capable vector. After an incubation period of 5-10 days, the infected mosquito can transmit virus for the rest of its life span (2 weeks to 1 month). Aedes albopictus is colder tolerant than Aedes aegypti, so it can survive and transmit virus in the more temperate regions of the United States and Europe.
The global incidence of dengue has increased dramatically in the last several decades, with an estimated 40%-50% of the world's population in 128 countries at risk. [2, 3, 4]

Today, severe dengue largely affects Asian and Latin American countries, where it is a leading cause of hospitalization and death. The World Health Organization (WHO) ranked dengue as one of the top ten threats to global health in 2019 [5].

Initial dengue infection may be asymptomatic (50%-90%) [6], may result in a nonspecific febrile illness, or may produce the symptom complex of classic dengue fever (DF). Classic dengue fever is marked by rapid onset of high fever, headache, retro-orbital pain, diffuse body pain (both muscle and bone), weakness, vomiting, sore throat, altered taste sensation, and a centrifugal maculopapular rash, among other manifestations. The severity of the pain led to the term breakbone fever to describe dengue.

A small percentage of persons who have previously been infected by one dengue serotype develop bleeding and endothelial leak upon infection with another dengue serotype. This syndrome is termed severe dengue (reclassified in 2009 by the WHO, previously referred to as dengue hemorrhagic fever and dengue shock syndrome).

Severe dengue has also been termed dengue vasculopathy. Vascular leakage in these patients results in hemo concentration and serous effusions and can lead to circulatory collapse. This, in conjunction with severe hemorrhagic complications, can lead to a shock syndrome, which poses a greater fatality risk than bleeding per se [7].

Dengue virus transmission follows 2 general patterns: epidemic dengue and hyperendemic dengue. Epidemic dengue transmission occurs when dengue virus is introduced into a region as an isolated event that involves a single viral strain. If the number of vectors and susceptible pediatric and adult hosts is sufficient, explosive transmission can occur, with an infection incidence of 25-50%. Mosquito-control efforts, changes in weather, and herd immunity contribute to the control of these epidemics. Transmission appears to begin in urban centers and then spreads to the rest of the country [8]. This is the current pattern of transmission in parts of Africa and South America, areas of Asia where the virus has re-emerged, and small island nations. Travellers to these areas are at increased risk of acquiring dengue during these periods of epidemic transmission.

Hyperendemic dengue transmission is characterized by the continuous circulation of multiple viral serotypes in an area where a large pool of susceptible hosts and a competent vector (with or without seasonal variation) are constantly present. This is the predominant pattern of global transmission. In areas of hyperendemic dengue, antibody prevalence increases with age, and most adults are immune. Hyperendemic transmission appears to be a major risk for dengue hemorrhagic fever. Travelers to these areas are more likely to be infected than are travellers to areas that experience only epidemic transmission [9].

Because the signs and symptoms of dengue fever are nonspecific, attempting laboratory confirmation of dengue infection by serodiagnosis, reverse-transcriptase polymerase chain reaction (RT-PCR), or culture is important. Serodiagnosis is made on the basis of a rise in antibody titre in paired IgG or IgM specimens. Results vary depending on whether the infection is primary or secondary. Dengue is a reportable disease in the United States; known or suspected cases should be reported to public health authorities.

Dengue fever is usually a self-limited illness. Supportive care with analgesics, judicious fluid replacement, and bed rest is usually sufficient. Successful management of severe dengue requires intravascular volume replacement, with careful attention to fluid management and proactive treatment of hemorrhage. Admission to an intensive care unit is indicated for patients with severe dengue.

Severe dengue occurs less frequently than dengue fever but has a more dramatic clinical presentation. In most of Asia, where it first was described, severe dengue is primarily a disease of children. However, in the Americas, and more recently reported in Taiwan, severe dengue has an equal distribution in all ages.

Severe dengue typically begins with the initial manifestations of dengue fever. The acute febrile illness (temperatures ≤40°C), like that of dengue fever, lasts approximately 2-7 days. However, in persons with severe dengue, the fever reappears, giving a biphasic or saddleback fever curve.

Along with biphasic fever, patients with severe dengue have progressive thrombocytopenia, increasing hematocrit (20% absolute rise from baseline) and low albumin (signs of hemoconcentration preceding shock), more obvious hemorrhagic manifestations (>50% of patients have a positive tourniquet test), and progressive effusions (pleural or peritoneal). Lymphocytosis, often with atypical lymphocytes, commonly develops before defervescence or the onset of shock. Transaminase levels may be mildly elevated or present in the several thousands associated with hepatomegaly in those patients with acute hepatitis. Low fibrinogen and elevated fibrin split products are signs of disseminated intravascular coagulation. Severe metabolic acidosis and circulatory failure can occur.

The critical feature of severe dengue is plasma leakage. Plasma leakage is caused by increased capillary permeability and may manifest as hemoconcentration, as well as pleural effusion and ascites. Bleeding is caused by capillary fragility and thrombocytopenia and may manifest in various forms, ranging from petechial skin hemorrhages to life-threatening gastrointestinal bleeding.

Liver damage manifests as increases in levels of alanine aminotransferase and aspartate aminotransferase, low albumin levels, and deranged coagulation parameters (prothrombin time, partial thromboplastin time). In persons with fatal dengue hepatitis, infection was demonstrated in more than 90% of hepatocytes and Kupffer cells with minimal cytokine response (tumor necrosis factor [TNF]–alpha, interleukin [IL]–2). This is similar to that seen with fatal yellow fever and Ebola infections [10].

As the term implies, severe dengue shock is essentially dengue hemorrhagic fever with progression into circulatory failure, with ensuing hypotension, narrow pulse pressure (< 20 mm Hg), and, ultimately, shock and death if left untreated. Death may occur 8-24 hours after onset of signs of circulatory failure. The most common clinical findings in impending shock include hypothermia, abdominal pain, vomiting, and restlessness.

Dengue virus infection has emerged as a notable public health problem in recent decades in terms of the mortality and morbidity associated with it. Dengue is endemic in many parts of India and epidemics are frequently reported from various parts of India and abroad. The case fatality rate in patients with dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) can be as high as 44%. Hence based on above findings the present study was planned for Study of Sero prevalence of Dengue Infection in Clinically Suspected Cases of Dengue at NMCH, Patna.

Methodology

The present study was planned in Department of Microbiology, Nalanda Medical College and Hospital Patna, Bihar, India. The study was conducted from August2019 to December 2019. In the present study 909 patients of suspected dengue cases were evaluated.

A single blood sample approximately 2-3 ml was collected from each patient in a plain vaccutainer tube with all aseptic precautions. Serum was subjected to NS1, IgG and IgM capture ELISA.

Detection of NS1, IgM and IgG Antibodies was performed by the ELISA (EnzymeLinked Immunosorbent Assay) test using kit provided by National Institute of Virology (NIV), Pune. The Positive control and Negative control from the kit were put up with the test samples as per the kit literature provided. The test was a solid phase ELISA based on 'Direct Sandwich' principle. A positive reaction was indicated by a yellow colour which was precisely read at 450nm spectrophotometrically by an ELISA reader the cut-off value (COF) was calculated using the formula as per the recommendation of the manufacturer. Further samples were interpreted as Nonreactive, Equivocal and Reactive.

All the patients were informed consents. The aim and the objective of the present study were conveyed to them. Approval of the institutional ethical committee was taken prior to conduct of this study.

Results & Discussion

Out of the 909 samples tested, 356 samples were positive for either one or more of the three markers i.e. NS1 antigen, IgM and IgG antibody. Of the 356 samples; 182(51.12%) were positive for NS1 antigen only, 29 (8.14%) for IgM only which indicated recent dengue infection while 56 (15.73%) for IgG only which showed past infection with dengue.

More than one marker was detected in the remaining 89 (25%) samples. Primary dengue was detected in 224 (33%) cases and secondary dengue was detected in 132 (37%) cases.

Dengue infection presents with non-specific fever that mimics other viral illnesses. To prevent the outbreaks it is necessary to diagnose the dengue virus infection as early as possible. The diagnostic modalities include virus isolation, RNA detection, NS1 antigen detection and IgM or IgG antibody detection For a long time, the diagnosis of dengue infection was based on detection of dengue specific IgM / IgG antibody. Ig M and Ig G antibody detection tests which are used in acute primary and secondary infections can cross react with other flavivirus group and yield a false positive result. On the contrary NS1 antigen being specific for dengue does not cross react with other flavivirus group excluding the secondary cases where immune complex formation occurs. The new parameter for diagnosis of dengue infection, NS1 antigen, is detectable from day one of fever in both primary and secondary infections and declines to undetectable levels by 5-6 days.

Dengue is caused by dengue virus, a Flavivirus in the family of Togaviridae. During acute dengue infections when IgM is not readily detectable, NS1 antigen-based ELISA shall be considered as an important diagnostic tool. There are four known virus serotypes (DEN 1, DEN 2, DEN 3, and DEN 4) [11]. All these four serotypes share common geographical and ecological niche. All the four serotypes are now spreading in Asia, Africa and American continents. Their infection, transmission, different symptoms and pathogenesis are causing severe challenge to public health. The epidemiology of dengue in Indian subcontinent is very composite and distorted over time. Dengue was previously supposed to be an urban disease as most cases were reported from bigger cities. But from last decade there are many outbreaks from rural areas of southern and western India [12, 13].

The detection of IgM antibody to dengue virus by ELISA has become one of the laboratory's essential methods for the diagnosis of dengue virus infection. Anti-dengue IgM antibody is produced rapidly during primary and secondary infections.

Early diagnosis of dengue virus infection is important for treatment and aversion of complications like dengue shock syndrome (DSS) and dengue haemorrhagic fever (DHF). Dengue virus specific IgM antibodies appear as early as three days of dengue viral fever and can persist for 30-60 days, whereas IgG antibodies appear at about seventh day, peak at 2-3 wk and persist for life [14].

Based on the endemicity of dengue, World Health Organization (WHO) has kept India under category A, considering the dengue as a major public health problem, leading cause of hospitalization and death among children, hyperendemicity with all four serotypes circulating in urban areas, and spread to rural areas [15]. The risk of dengue has shown an increase in recent years due to rapid urbanization, life style changes and poor water management including improper water storage practices in urban, peri-urban and rural areas, leading to proliferation of mosquito breeding sites [16].

Dengue is an important emerging disease of the tropical and subtropical regions today. Dengue infection has been known to be endemic in many parts of India for over two centuries as a benign and self-limited disease. Epidemics of dengue are increasing in frequency. Detection of all four dengue serotypes in India has now rendered India hyperendemic [17].

Table 1: Patients Testing

Parameters	No. of Cases
Total Patients Analysed	909
No. of Positive patients	356
Males	190
Females	166

Table 2: Age group

Age group	No. of Cases
Less than 20 years	34
21-30 years	60
31-40 years	93
41-50 years	51
51-60 years	59
More than 60 years	59
Total	356

Dengue has traditionally been held to be a disease of high population density tropical urban areas [20, 21]. However, increasing reports of dengue cases and outbreaks from rural areas were reported from northern, southern and western India [22, 24]. The findings pertaining to the present study provide similar picture with other parts of the country. The epidemics of dengue have been commonly associated with the rainy season [25]. In a study conducted in Lucknow, India and Pakistan, it was observed that dengue transmission

occurred round the year with peak incidence in the postmonsoon season [18, 19]. Similarly, these studies found the highest proportion of dengue positive patients during post monsoon season. In the present study also most of the cases were found to report during post monsoon season with maximum number of cases from September to December.

The age group of 15–30 years was highly affected with dengue which is consistent with the outbreak in Delhi in 2003 [26]. In some parts of the world, it is mainly a pediatric public health problem [27]. It is attributed to the changes in locations where disease transmission takes place. The higher prevalence of dengue infection was noted among male patients than female patients unlike other reports in which both the sexes were equally affected [28].

Dengue fever is most common and important public health problem in developing countries like India, it can present with various acute clinical symptoms and causes major morbidity and mortality compared to any other viral infections. Therefore, a high index of interpretation is required for these patients to be diagnosed. Our results propose that NS1 and IgM-capture ELISA is very useful and effective method for the diagnosis of acute dengue infection. Therefore, this IgM antibody detection test will be helpful in diagnosis of dengue infection early so that the morbidity and mortality can be monitored and thus we conclude that this serological test have crucial part in the early diagnosis of dengue infection.

Laboratory-based active surveillance systems are needed to complement the current passive surveillance and control programs. Regular sentinel surveillance and sample surveys during interepidemic periods are also necessary to detect and monitor sudden increases in the numbers of dengue cases or changes in the predominant serotypes which usually precede major outbreaks. New molecular diagnostic techniques, such as RT-PCR, are particularly useful in this context, their speed and sensitivity enabling the rapid detection of increased viral circulation or changes in predominant serotypes.

Conclusion

The data generated from the present study concludes that Dengue cases were more during September to December in the post monsoon season which is useful to plan special preventive strategies. The study draws attention toward the male, young adult age group. All suspected cases of dengue must be monitored for all the three parameters i.e. NS1 antigen, IgM and IgG antibody to differentiate between primary and secondary infection as the possibility of DHF and DSS are more in secondary infection.

References

1. World Health Organization. Dengue and severe dengue fact sheet. WHO. Available at http://www.who.int/mediacentre/factsheets/fs117/en/. April 2017; Accessed: September 28, 2017.
2. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, *et al*. The global distribution and burden of dengue. Nature. 2013; 496(7446):504-7. [Medline].
3. Brady OJ, Gething PW, Bhatt S, Messina JP, Brownstein JS, Hoen AG, *et al*. Refining the global spatial limits of dengue virus transmission by evidence-based consensus. PLoS Negl Trop Dis. 2012; 6(8):e1760.
4. Wilson ME, Chen LH. Dengue: update on epidemiology. Curr Infect Dis Rep. 2015; 17(1):457.
5. Ten threats to global health in. World Health Organization, 2019. Available at https://www.who.int/emergencies/ten-threats-to-global-health-in-2019. 2019; Accessed: Feb 2, 2019.
6. Kyle JL, Harris E. Global spread and persistence of dengue. Annu Rev Microbiol, 2008. 62:71-92.
7. 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.
8. Gubler DJ. Cities spawn epidemic dengue viruses. Nat Med. 2004; 10(2):129-30.
9. Wilder-Smith A, Gubler DJ. Geographic expansion of dengue: the impact of international travel. Med Clin North Am. 2008; 92(6):1377-90.
10. de Macedo FC, Nicol AF, Cooper LD, Yearsley M, Pires AR, Nuovo GJ, *et al*. Histologic, viral, and molecular correlates of dengue fever infection of the liver using highly sensitive immunohistochemistry. Diagn Mol Pathol. 2006; 15(4):223-8.
11. Maxcy-Roseneau-Last. Public health and preventive medicine. Mc Graw Hill pub, 2008; 15:350-51.
12. Gupta N, Srivastava S, Jain A, Chaturvedi UC. Dengue in India. Indian J Med Res, 2012; 136:373-90.
13. Tripathi P, Kumar R, Tripathi S, Tambe JJ, Venkatesh V. Descriptive epidemiology of dengue transmission in Uttar Pradesh. Indian Pediatr, 2008; 45:315-8.
14. Vijayakumar TS, Chandy S, Sathis N, Abraham M, Abraham P, Sridharan G, *et al*. Is dengue emerging as a major public health problem? Indian J Med Res, 2005; 121:100-7.
15. Comprehensive guidelines for prevention and control of dengue and dengue hemorrhagic fever. Rev edn. New Delhi: Regional office of SEAR, World Health Organization, 2011. Available from: http://apps.searo.who.int/pds_docs/B4751.pdf (Accessed on November 10, 2014).
16. Park K. The dengue syndrome. In: Park K, editor. Parks text book of preventive and social medicine. XXII edn. Jabalpur (India): M/s Banarasidas Bhanot Publishers, 2013, 224-32.
17. Maheshwari D, Dadhich D, Saxena N. Seroprevalence of dengue in Kota, Rajasthan: a study at a tertiary care hospital. Journal of Evolution of Medical and Dental Sciences, 2015; 4:821-5.
18. Khan E, Kisat M, Khan N, Nasir A, Ayub S, Hasan R. Demographic and Clinical Features of Dengue Fever in Pakistan from 2003–2007: A Retrospective CrossSectional Study. Plos ONE. 2010; 5(9):E12505.
19. Tripathi P, Kumar R, Tripathi S, Tambe JJ, Venkatesh V. Descriptive Epidemiology of Dengue Transmission in Uttar Pradesh. Indian Pediatrics, 2008; 45:315-8.
20. Teixeira MG, Barreto ML, Costa MCN, Denize L, Ferreira A, Vasconcelos PFC, *et al*. Dynamics of Dengue Virus Circulation: A Silent Epidemic in a Complex Urban Area. Tropical Medicine and International Health, 2002; 7:757-62.
21. Pavri KM. Ecology of Mosquito Borne Viruses in India and Southeast Asia. In: Loutit MW, Miles JAR. Eds. Microbial Ecology. Berlin, 1978.
22. Kumar A, Sharma SK, Padbidri VS, Thakare JP, Jain DC, Datta KK. An Outbreak of Dengue Fever in Rural Areas of Northern India. Journal of Communicable

Disease, 2001; 33:27-81.
23. Norman G, Theodre A, Joseph A. An Insular Outbreak of Dengue Fever in a Rural South Indian Village. Journal of Communicable Diseases, 1991; 23:185-90.
24. Ilkal MA, Dhanda V, Hassan MM, Mavale M, Mahadev PV, Shetty PS, *et al*. Entomological Investigations During Outbreaks of Dengue Fever in Certain Villages in Maharashtra State. Indian Journal Medical Research, 1991; 93:174-8.
25. Keating J. An Investigation into the Cyclical Incidence of Dengue Fever. Social Science and Medicine, 2001; 53:1587-97.
26. Gupta E, Dar L, Narang P, Srivastava VK, Broor S. Serodiagnosis of dengue during an outbreak at a tertiary care hospital in Delhi. Indian J Med Res, 2005; 121:36-8.
27. Gubler DJ. Dengue and Dengue haemorrhagic fever. Clin Microbiol Rev, 1998; 11:480-96.
28. Mehendale SM, Risbud AR, Rao JA, Banerjee K. Outbreak of Dengue fever in rural areas of Parbhani district of Maharashtra, India. Indian J Med Res, 1991; 93:6-11.
29. Prasad A, Kumar A. Susceptibility status of dengue vector Aedes aegypti (L.) against various larvicides and insecticides in Udaipur district of Southern Rajasthan, India. India. Int J Entomol Res. 2019;5(1):74-7.