Original Article

Dengue in children and young adults, a cross-sectional study from the western part of Uttar Pradesh

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Abstract

Background: Dengue has emerged as a major public health problem in India. It presents more aggressively among younger age groups as compared to adults. Therefore, it necessitates the accurate estimation of prevalence in younger age groups. **Materials and Method:** Of all the 1026 clinically suspected cases of dengue up to the age of 18 years were enrolled in this study and grouped into four age groups (Group I - <0 to 1 year, Group II- 1 to 6 years, Group III- 7 to 12 years, and Group IV- 13 to 18 years). Their blood samples were aseptically collected from different clinical departments and were submitted to the Viral Research and Diagnostic Laboratory (VRDL), Department of Microbiology during the outbreak of 2016–17. Serum was separated and processed for dengue Non Structural Protein 1 antigen (NS1 Ag)and Immunoglobuline M antibody (IgM Ab) enzyme-linked immunosorbent assay (ELISA). All the relevant variables like age, sex, and demographic profile were recorded and statistically analyzed. **Results:** A total 295 of the 1026 cases were detected positive for dengue either by NS1 Ag or IgM Ab ELISA. The results show the susceptibility to dengue being increased in the order of age Group I to IV. We analyzed the outbreak of year 2016 and 2017, of these 159/483 (33%) cases and 136/543 (25%) cases, respectively, were found seropositive during these years. The months of September, October, and November are more prone to dengue infection. **Conclusion:** Group III and IV were more susceptible to dengue fever (DF). The months of postmonsoon season are more favorable for spread of dengue among different age groups of the population.

Keywords: Dengue, IgM Ab, NS1 Ag

Introduction

Dengue fever (DF) is an important vector-borne emerging and re-emerging infection and has become a major public health problem of tropical and subtropical regions of the world.[1] The World Health Organization (WHO) has estimated that 2.5 billion population and 124 countries are at the risk of dengue infection with over 100 million cases and 30,000 estimated deaths worldwide.[1] In most of the endemic countries, it regularly causes epidemics after a period of every 2–4 years, and India has been witnessing such outbreaks each year for the last one decade.[2,3] Over a period of time, DF has evolved clinically, thus presenting in a variety of clinical presentations showing from mild or moderate disease called classical to sometimes resulting in severe forms, such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) in children and adults, both. The clinical presentation like high-grade fever, petechial rashes, abdominal pain, severe headache, and hepatomegaly are common in infants, whereas edema of the lower extremities, vomiting, retro-orbital puffiness, and seizures are common clinical signs and symptoms in children.[4]

It is estimated that over 50 million cases of severe DF occur annually in Asian countries. Globally, the index of severity has increased with increased infectivity since the last decade and our country is contributing a large number of cases because of the high susceptibility and favorable climatic conditions.[5,6] The disease has more severe presentations with the involvement of children of different age groups; therefore, if the condition

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is undiagnosed or untreated, the mortality of DF would be significantly increased. Thus, prompt diagnosis and management will help in reducing mortality, especially in children.[6-8] These facts are supported by studies, one from Ludhiana, India that reported that 81 children with DHF were hospitalized during the epidemic in 2005–2006 and another study from Chennai, India that reported that 20% of the total dengue cases were detected in infants during an outbreak of October 2012 to December 2012.[4,9]

Dengue-specific Non Structural Protein 1 antigen (NS1 Ag) and Immunoglobuline M antibody (IgM Ab) Enzyme-Linked Immunosorbent Assay (ELISA) are principal diagnostic modalities in endemic countries because of their cost-effectiveness and higher sensitivity and specificity.[10,11] Therefore, the present study aims to determine the prevalence of dengue in this region and comparative evaluation of these ELISAs in terms of their diagnostic efficacy used either singly or in combination with the different age groups of children.

Material and Method

Setting and clinical samples

It is a descriptive cross-sectional study conducted among children admitted to different clinical departments of our tertiary care hospital with symptoms related to classical dengue fever as manifested in the acute phase of the illness (1–6 days). The cases have been grouped into four age groups defined as Group I (<01 years), Group II (1–6 years), Group III (7–12 years), and Group IV (13–18 years). A total of 1026 blood samples were aseptically collected and transported to the (VRDL), Department of Microbiology during the outbreaks of 2016 and 2017. Serum was separated aseptically and one-half was immediately processed for ELISA (NS1 Ag and IgM Ab) and second half was stored at –80°C for further processing.

ELISA

The diagnosis of dengue was confirmed by two ELISAs, namely, NS1Ag (Qualisa® Dengue NS1 Qualpro Diagnostics Pvt. Ltd., Goa, India) and IgM antibody capture (MAC) (Microlisa® IgM, J. Mitra and Co, New Delhi). All the sera samples were subjected to both tests the same day as per the manufacturer's instructions as briefly described below.

NS1 ELISA

To begin, 50 µL sample diluent was added to each well and 100 µL of negative and positive controls were added, followed by serum samples in corresponding wells. The plate was incubated for 30 min. at 37°C and then washed and dried for removing any unwanted and/or unbound antigens. Further 100 µL of the conjugate was added to each well and incubated for 60 min at 37°C. The plate was again washed and dried followed next by the addition of 100 µL of substrate and further incubation for 15 min in dark at room temperature. Finally, 100 µL of stop solution was added and absorbance was read at 450 nm (Alere AM 2100).

IgM (MAC) ELISA

A 100 µL of diluted serum sample (1:100) and 100 µL of negative and positive controls were added to corresponding wells and incubated at 37°C for 60 min. The plate was washed five times and 100 µL of the conjugate was added and incubated for 60 min at 37°C. Washing was done again and 100 µL of the substrate was added incubated in dark for 30 min at 37°C. Finally, 100 µL of stop solution was added and absorbance read at 450 nm.

Analysis of results

The result analysis was done with the Statistical Package for the Social Sciences (SPSS) software, version 22.0 (IBM Corp., Armonk, New York, USA). The continuous variables were summarized as mean and standard deviation (SD). Descriptive statistics were used to calculate all the relevant variables.

Ethics approval

University Ethics Committee "Ref. No. 408 UPUMS/ Dean/2018-19 E.C. No. 2017/82" 05/06/2017 approved this study.

Results

Of the total 1026 samples tested, 295 (28.75%) cases were confirmed for dengue by either NS1 Ag or IgM Ab ELISA method. The case distribution among different age groups is as following, Group I- 0.5% ($N = 06$), Group II- 12.20% ($N = 36$), Group III- 25.76% ($N = 76$), and Group IV - 60% ($N = 177$). Males accounted for 189 (64%) of the total cases while the remaining 106 (36%) were females. The means ± SD of age 12.85 ± 5 years were noted in all dengue seropositive cases. The prevalence of dengue shows an increasing trend from age Group I to IV [Figure 1]. Of the two ELISAs, NS1 Ag ELISA detected maximum number of cases i.e. 227, and the IgM Ab ELISA could detect only 165 cases, while 97 cases were detected by both antigen and antibody ELISAs [Figure 1]. The high NS1 antigen positivity was significant ($P < 0.0001$) as against IgM antibody detection.

The outbreak analysis of the year 2016 and 2017, shows that 159/483 (33%) cases and 136/543 (25%) cases, respectively, were found seropositive during these years. The disease burden of the outbreak year 2016 was slightly higher with significant

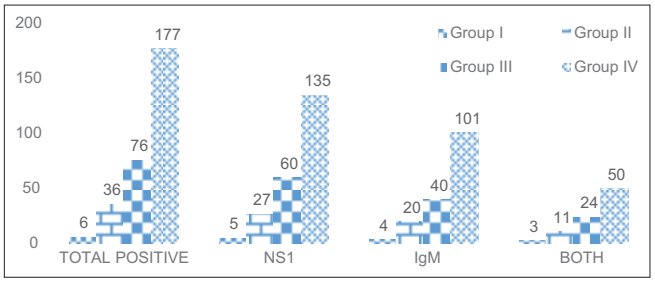

Figure 1: Distribution of age group wise NS1 and IgM seropositive cases at *X*-axis and total cases at *Y*-axis ($N = 295$)

difference ($P = 0.03$) over the year 2017 [Table 1]. The peak burden of disease was seen in the months from September to November in both outbreak seasons. In the outbreak of 2016, the first case was reported in the month of August, while in 2017, the first case was reported in the month of July. In the monthly distribution of these cases between both outbreak years, September of 2016 was significantly higher ($P < 0.0001$) as against the same month of 2017. While the month of November 2017 showed slightly higher significance ($P = 0.02$) in comparison to November 2016. [Table 1]

Discussion

Over the last few decades, dengue has emerged as a major public health problem in the Indian subcontinent and its neighboring countries.[2,12] The prevalence of dengue and its associated mortality has significantly increased in our country over the last few years.[3,13] There is a scarcity of data, as shown by various surveillance systems, pertaining to the prevalence among different age groups of affected children. There are few studies that have reported the data of the small sample size.[6,14,15] In view of the above facts, the present study has been done in a way that it deals with large sample size to know the actual disease prevalence among children. The study spans over two seasonal years (2016 and 2017) as reflected by the results—295/1026 (28.75%) of dengue cases were detected during these two years. The rapid increase in the number of cases during the year 2016–17 became a public health concern in northern India as the majority of those affected were in age Group IV (13–18 years) and second common affected age was Group III (7–12 years) [Table 1]. Indeed, these groups of children were schoolgoing, who might have borne the brunt because of lack of awareness regarding protective measures. Numerous authors detected that the maximum numbers of cases were seen in the age group of >11 years (34.02%) and the least affected age group was infants.[12,14,16] More involvement of adolescents can be explained by diurnal adaptation of the *Aedes* mosquito in stored water. These children play in the open fields and it makes them prone to an attack from *Aedes* mosquitoes.[14] It was also observed that males were more commonly affected than females; it might be because males were more active in outdoor games compared with females of their age groups. The similar finding was observed by other authors, e.g. Mishra *et al.* 2016[14] found a significant difference in male: female ratio (3.4:1) and the results of Shah *et al.* 2006[16] also supported the present results as their study shows more predilection for males. Therefore, the primary prevention for dengue has to be taken care of by families by using mosquito net while sleeping, mosquito repellents inside/outside home, and preventing access of mosquitoes to an infected person with fever. The family and community must be constantly made aware thus improving their knowledge and be proactive in preventing the spread of disease through campaign and social mobilization.

Diagnosing sporadic cases of dengue in an infant or very young child is a challenge to the clinician because of the absence of characteristic clinical symptoms. However, during an outbreak, the infection should be ruled out in all children presenting with fever to any healthcare facility.[17]

The dengue fever presents with a variety of symptoms with or without warning signs. Although most of the dengue cases are self-limited, some cases may vary in severity and deteriorate instead of recovering. Thus, primary diagnosis plays the most important role in disease management. The commonly identified features during febrile conditions, recognized as critical, are plasma leakage and severe bleeding, which warrants immediate medical attention.

The diagnosis of dengue is mainly based on serological detection, which includes NS1 Ag and IgM antibody ELISA. The NS1 Ag ELISA is more sensitive in the acute phase of the illness, especially in cases up to one week of onset, while the IgM assay is most effective between 4 to 15 days of onset.[11,18] In the present study, to maintain the accuracy and precisions in diagnosis, all samples were analyzed by both assays same day. Despite this, the NS1 came out as more effective assay, and it detected maximum of the cases (227) followed by IgM, which could detect 164 cases, although 97 cases were detected by both assays. Due to the higher positivity of NS1 Ag ELISA, the results of these are statistically significant over the IgM Ab ELISA. As NS1 of dengue virus is a highly conserved glycoprotein and produced in both a membrane-associated form and secreted form, the NS1 secreted during the early stage from infected mammalian cells stimulates a strong humoral response.[19] It is also produced in higher concentrations during primary and secondary dengue infection up to 1–7 days from the onset of illness.[20]

The annual prevalence during the outbreak year 2016 was 33%, and in 2017, it decreased to 25% among the total cases. However, data analysis shows that a higher positivity in 2016 is statistically significant over the prevalence of 2017. It might be because of severity in the nature of an outbreak in the year 2016 and rising awareness regarding dengue prevention from various types of electronic and print media. Our results, which are supported

Table 1: Monthly distribution of total seropositive dengue fever cases

Years	Total samples	Positive samples	Monthwise distribution of cases					
			July	Aug	Sept	Oct	Nov	Dec
2016	483	159*	0	6	54*	66	28	5
2017	543	136	1	5	19	55	54*	2
P^a		0.03S	>.05NS	>.05NS	<.0001S	>.05NS	0.02S	>.05NS

[a]*P*-value<0.05 is considered is to be statistically significant. S=Significant, NS=nonsignificant

by the results of the National Vector Borne Disease Control Programme (NVBCP), India, showed that during the outbreak of the year 2016, the total affected population was twice in proportion to outbreak 2017 in Uttar Pradesh India.[3]

The monthly distribution of cases has also been analyzed in this study, which shows a higher incidence from September onward to November months in both years. The months of September–October 2016 and October–November 2017 had maximum number of cases in both epidemic years. The outbreak of 2016 was most severe in terms of high incidence, and it was statistically significant in comparison with 2017 ($P = 0.03$) [Table 1]. Many authors have also reported that the months from September to November were peak session during the outbreaks each year.[21,22] It can be explained by the fact that monsoon and the postmonsoon seasons offer the most favorable conditions for mosquito breeding in northern India.

Thus, this study concludes that in our country dengue has a periodicity, and this cycle has been occurring regularly each year since 2010. Due to the increase in the number of dengue cases, it correlates with high morbidity and mortality in children, especially schoolgoing children and adolescents who are more prone to infection. In addition, the combination of NS1 Ag and IgM Ab ELISA can help in early and accurate diagnosis and management of dengue fever.

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Conflicts of interest

There are no conflicts of interest.

References

1. World Health Organization and the Special Programme for Research and Training in Tropical diseases (TD. Dengue: Guidelines for diagnosis, treatment, prevention, and control" 2009. Available from: http://www.who.int/mediacentre/factsheets/fs117/en/, accessed on April 11, 2019.
2. Suleman M, Lee HW, Zaidi SS, Alam MM, Nisar N, Aamir UB, *et al.* Preliminary seroepidemiological survey of dengue infections in Pakistan, 2009-2014. Infect Dis Poverty 2017;6:48.
3. National Vector Born Disease Control Programme, Director General of Health Services. Ministry of Health and Family welfare. Dengue Cases and Deaths in the Country since 2010. Available from: http://nvbdcp.gov.in/den-cd.html. [Last accessed on 2018 Apr 11].
4. Dhooria G, Bhat D, Bains H. Clinical profile and outcome in children of dengue hemorrhagic fever in North India. Iran J Pediatr 2008;18:222-8.
5. Murhekar MV, Kamaraj P, Kumar MS, Khan SA, Allam RR, Barde P, *et al.* Burden of dengue infection in India, 2017: A cross-sectional population based serosurvey. Lancet Glob Health 2019;7:1063-73.
6. Palanivel H, Nair S, Subramaniyan A, Ratnam PV, Kanungo R. Dengue virus infection: Need for appropriate laboratory tests for diagnosis and management of the condition in children during an outbreak. Indian J Pathol Microbiol 2015;58:328-31.
7. Rose W, Sindhu KN, Abraham AM, Kang G, John J. Incidence of dengue illness among children in an urban setting in South India: A population based study. Int J Infect Dis 2019;84:15-8.
8. Witayathawornwong P. Dengue hemorrhagic fever in infancy at Petchabun Hospital, Thailand. Southeast Asian J Trop Med Public Health 2001;32:481-7.
9. Kabilan L, Balasubramanian S, Keshava SM, Thenmozhi V, Sekar G, Tewari SC, *et al.* Dengue disease spectrum among infants in the 2001 dengue epidemic in Chennai, Tamil Nadu, India. J Clin Microbiol 2003;41:3919-21.
10. Chan HB, How CH, Ng CW. Definitive tests for dengue fever: When and which should I use? Singapore Med J 2017;58:632-5.
11. Chaterji S, Allen JC Jr, Chow A, Leo YS, Ooi EE. Evaluation of the NS1 rapid test and the WHO dengue classification schemes for use as bedside diagnosis of acute dengue fever in adults. Am J Trop Med Hyg 2011;84:224-8.
12. Tissera H, Amarasinghe A, De Silva AD, Kariyawasam P, Corbett KS, Katzelnick L, *et al.* Burden of dengue infection and disease in a pediatric cohort in urban Sri Lanka. Am J Trop Med Hyg 2014;91:132-7.
13. Gupta N, Srivastava S, Jain A, Chaturvedi UC. Dengue in India. Indian J Med Res 2012;136:373-90.
14. Mishra S, Ramanathan R, Agarwalla SK. Clinical profile of dengue fever in Children: A study from Southern Odisha, India. Scientifica 2016;2016:6391594.
15. Tripathi P, Kumar R, Tripathi S, Tambe JJ, Venkatesh V. Descriptive epidemiology of dengue transmission in Uttar Pradesh. Indian J Pediatr 2008;45:315-8.
16. Shah GS, Islam S, Das BK. Clinical and laboratory profile of dengue infection in children. Kathmandu Univ Med J 2006;4:40-3.
17. Anderson KB, Chunsuttiwat S, Nisalak A, Mammen MP, Libraty DH, Rothman AL, *et al.* Burden of symptomatic dengue infection in children at primary school in Thailand: A prospective study. Lancet 2007;369:1452-9.
18. Palomares-Reyes C, Silva-Caso W, del Valle LJ, Aguilar-Luis MA, Weilg C, Martins-Luna J, *et al.* Dengue diagnosis in an endemic area of Peru: Clinical characteristics and positive frequencies by RT-PCR and serology for NS1, IgM, and IgG. Int J Infect Dis 2019;81:31-7.
19. Gowri SS, Dhananjeyan KJ, Paramasivan R, Thenmozhi V, Tyagi BK, John Vennison S. Evaluation and use of NS1 IgM antibody detection for acute dengue virus diagnosis: Report from an outbreak investigation. Clin Microbiol Infect 2012;18:8-10.
20. Dussart P, Labeau B, Lagathu G, Louis P, Nunes MR, Rodrigues SG, *et al.* Evaluation of an enzyme immunoassay for detection of dengue virus NS1 antigen in human serum. Clin Vaccine Immunol 2006;13:1185-9.
21. Bhattacharya N, Mukherjee H, Naskar R, Talukdar S,

Das G, Pramanik N, *et al.* Serological diagnosis of dengue in laboratory practice in Kolkata. Indian J. Med Microbiol 2014;32:277-80.

22. Ray A, Mohta S, Soneja M, Jadon R, Wig N, Sood R. Clinical spectrum and outcome of critically ill hospitalized patients with acute febrile illness and new-onset organ dysfunction presenting during monsoon season. Drug Discov Ther 2019;13:101-7.