International Journal of Research in Medical Sciences
Kumar AS et al. Int J Res Med Sci. 2019 May;7(5):1558-1561
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20191635

Original Research Article

Increasing number of secondary dengue cases: a concern

Kumar S. Abhishek[1]*, Oves Siddiqui[2], Anita Chakravarti[3]

[1]Department of Microbiology, All India Institute of Medical Sciences, Jodhpur, Rajasthan, India
[2]Department of Microbiology, Maulana Azad Medical College, New Delhi, India
[3]Department of Microbiology, SGT Medical College Hospital and Research Institute, Gurugram, Haryana, India

Received: 17 December 2018
Accepted: 29 January 2019

***Correspondence:**
Dr. Kumar S. Abhishek,
E-mail: get.abhi1904@gmail.com

ABSTRACT

Background: Dengue fever is caused by mosquito borne arbovirus of family *Flaviviridae*, *Aedes agypti* as the principle vector. In the recent past Delhi has witnessed several outbreaks affecting thousands of individuals and many of them get re-infected during subsequent years forming a bulk of secondary dengue cases putting them at risk of developing severe dengue.
Methods: A total of 150 serum samples from suspected dengue cases were tested for dengue fever by NS-1 antigen and IgM antibody enzyme-linked immunosorbent assay (ELISA) followed by categorization into primary and secondary dengue using IgG avidity ELISA.
Results: Out of total 150 clinically suspected dengue cases, 56 were positive either by Dengue NS-1 antigen or dengue IgM antibody or both. On the basis of dengue IgG avidity ELISA among 56 diagnosed dengue cases, 30 (53.57%) were found to be of secondary dengue.
Conclusions: There is increasing trend of dengue cases in Delhi since past one decade. Being hyper-endemic area for dengue, more than 25% population have been reported to have past infection of dengue. Due to increased prevalence and simultaneous circulation of more than one serotypes, number of secondary dengue cases is also increasing. Since majority of severe dengue cases are associated with secondary dengue, early diagnosis and treatment can significantly reduce the fatal outcome. Thus, avidity testing for IgG antibody becomes an important tool.

Keywords: ELISA, Flaviviridae, IgG avidity, IgM ELISA, NS-1, Severe dengue

INTRODUCTION

Dengue fever is caused by dengue virus a member of Flaviviridae and is primarily transmitted by *Aedes agypti*. Dengue fever presents with a wide spectrum of signs and symptoms ranging from an asymptomatic case to severe dengue in form of dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS).[1,2] Dengue virus has four serotypes which are referred as DENV1, DENV2, DENV3 and DENV4 having an enveloped positive sense single stranded RNA.[1] Though the fifth variant DENV5 has been isolated but its presence in India is not yet documented.[3] In the recent past dengue has become a concern worldwide especially in tropical countries including India. Delhi is one of the hyper-endemic regions and has witnessed several outbreaks in last decade with 2015 being the worst of them. As per the government data more than 15000 confirmed dengue cases with 60 deaths occurred in Delhi alone during 2015 outbreak.[4]

The exact pathogenesis for development of severe dengue is not yet very clear. Amongst various hypotheses, antibody dependent enhancement (ADE) during secondary dengue with different serotype is the most accepted one. It results due to the presence of circulating non-neutralizing antibody from the past dengue infection of another serotype.[5] All the four dengue serotypes are found to circulate in Delhi with predominance of one or more serotypes. Co-dominance of DENV2 and DENV3 was seen during 2012 while DENV2 dominated during 2013 and 2015.[6,7] About 25% population in Delhi has been reported to have past dengue infection and due to this changing trend of circulating dengue serotypes, chances of acquiring secondary infection with different serotype is very high.[8]

Thus, merely diagnosing the dengue infection either by NS-1 antigen and/or IgM antibody detection is not enough and there should be a routine trend of diagnosing primary and secondary dengue infection even if serotyping facility is not available as it requires much expertization and technical support. Avidity IgG antibody testing is a simple and useful tool to diagnose primary and secondary dengue infection. This study focuses on the use of avidity IgG dengue antibody testing for early diagnosis of secondary dengue so that the future development of severe form of dengue could be prevented well in time.

METHODS

This is a cross sectional study, conducted at the Department of Microbiology, Maulana Azad Medical college and associated Lok Nayak Hospital between July to September 2015. Blood samples from 150 clinically suspected dengue adult patients attending the Medicine outpatient department (OPD) were collected in a plain vial and tested for dengue. Dengue infection were confirmed by detection of NS-1 antigen or/and IgM antibody using dengue NS-1 Ag Microlisa (J. Mitra and Co.) and NIV Den IgM Capture ELISA kits respectively as per manufacturer's instructions. All the confirmed dengue samples were further tested for IgG avidity ELISA to determine the numbers of primary and secondary dengue.

Panbio IgG ELISA kit with an additional step as described for IgG antibody for cytomegalovirus was used.[9] Serum samples were tested in duplicates, after the initial incubation and washing an additional step with addition 100µl of 8 M urea in PBS was added to the second well of each duplicate samples while 100µl of PBS was added to the first well. Plates were incubated for 5minutes at room temperature and washed twice with the wash buffer. Rest of the steps were followed as per the manufacturer's instructions.

Calculation of avidity index was done by dividing OD value of well exposed to urea with OD value of corresponding well not exposed to urea. Avidity index of less than 0.8 were considered as primary dengue while avidity index with more than or equal to 0.8 were considered as secondary dengue. All the confirmed dengue cases were further classified in to non-severe and Severe dengue on the basis of clinical presentations and biochemical profile.

RESULTS

A total of 150 samples were tested and out of them 56 were positive for either NS-1 antigen and/or IgM antibody with positivity rate of 37.3% (56/150) (Table 1).

Table 1: Dengue serology by enzyme-linked immunosorbent assay (ELISA).

Test result	NS-1 positive	NS-1 negative	Total
IgM positive	09	12	21
IgM negative	35	94	129
Total	44	106	150

Out of 56 confirmed dengue cases, 30 (53.57%) were found to be secondary dengue by avidity IgG ELISA.

Table 2: Distribution of primary and secondary dengue infection according to severity.

Dengue type	Non-severe dengue	Severe dengue	Total
Primary	22	04	26
Secondary	16	14	30
Total cases	38	18	56

On the basis of clinical manifestations and biochemical profile, 18 (32.15%) cases were of severe dengue and out of all these 18 severe dengue cases 14 (77.8%) alone were from secondary dengue (Table 2) and (Figure 1).

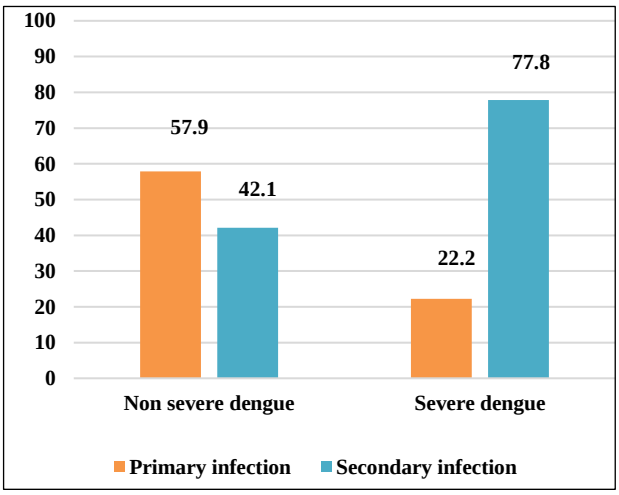

Figure 1: Percentage wise distribution of severe and non-severe dengue.

DISCUSSION

Delhi being a hyper-endemic state for dengue, almost every year a huge population is at risk of acquiring dengue infection especially during the monsoon and post monsoon period. Enhanced virus transmission during these period is probably due to high humidity, temperature and rainfall together with poor water drainage system which leads to rapid proliferation of mosquitoes.[10] Increasing city population and rapid urbanization plays an additional and important role.[11] With the increasing numbers of dengue cases each year, the number of individuals getting re-infected in subsequent year is also increasing leading to the bulk of secondary dengue cases. Various ELISA and rapid based tests are available for the diagnosis of dengue infection with varying sensitivity and specificity. Rapid tests are quite promising for the prompt diagnosis even at the bedside and have been as good as ELISA based diagnostic kits although ELISA is still superior when it comes to sensitivity and specificity.[12]

Each year the predominant circulating dengue serotype keeps on changing. During 1996 DENV1 predominated in Delhi and when authors look at the 2003 scenario, for the first time all the four dengue serotypes were reported with predominance of DENV2.[13] Presence of all the four serotypes, huge population and presence of vector that too in high number is of great concern. Though the pathogenesis of dengue is still unclear, antibody dependent enhancement plays an important role when the subsequent infection occurs with another serotype.[5]

Dengue infection triggers Th-1 and Th-2 type of immune response in body leading to secretion of various inflammatory and anti-inflammatory cytokines. The role of these cytokines in the pathogenesis of dengue is still needs to be studied in more depth.[14] It has been found that levels of various Interleukins (IL-4 and IL-10) increases with the severity of the disease.[15] As altered Interleukin levels predict the severe form of dengue, similarly concurrent testing for primary and secondary dengue can categorize the high risk cases. Since majority of the severe form of dengue are from the secondary infection and co-circulation of all the four serotypes further increases the probability of getting infected from the different serotype. Avidity IgG ELISA is an important and simple tool to diagnose primary and secondary dengue. de Souza VA et.al. also explained the utility of avidity IgG testing to discriminate between primary and secondary dengue virus infection.[16]

Dengue is a serious health problem in Delhi as the number of cases and population under risk are constantly increasing. Due to the co-circulation of all the four serotypes, risk of acquiring infection with different serotype is always a matter of concern. Each year the predominant serotype changes and thus the probability of getting infected with different serotype is high. Secondary dengue with different serotype is a potential risk for developing severe form of dengue. Prompt diagnosis and categorizing primary and secondary infection much before the signs and symptoms of severe dengue appears is the need of the time as the world is still looking for the appropriate anti-viral drug against dengue virus. Thus, avidity IgG ELISA can be a simple and useful tool to detect secondary infection and to alarm the treating physician to identify and start supporting treatment to prevent life threatening severe form of dengue.

The limitation of the study were the results obtained from the small sample size of the study may not always correlate with the real picture and burden of the disease. Further this study does not tell about the serotypes of the virus associated with the secondary cases.

ACKNOWLEDGEMENTS

Authors would like to thank Department of Microbiology, Maulana Azad Medical College, New Delhi, India.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: Not required

REFERENCES

1. Gubler DJ. Dengue and dengue haemorrhagic fever. Clin Microbiol Rev. 1998; 11:480-96.
2. Guzman MG, Kourf G. Dengue: An update. Lancet Infect Dis. 2002;2:33-42.
3. Mustafa MS, Rasotgi V, Jain S, Gupta V. Discovery of fifth serotype of dengue virus (DENV-5): A new public health dilemma in dengue control. Med J Armed Forces India. 2015;71(1):67-0.
4. National vector borne disease control programme, 2018. Available at: http://www.nvbdcp.gov.in/index4.php?lang=1&level=0&linkid=431&lid=3715. Accessed on 15 December 2018.
5. Halstead SB. Observations related to pathogenesis of dengue haemorrhagic fever. VI. Hypotheses and discussion. Yale J Biol Med. 1970;42:350-62.
6. Sharma P, Mittal V, Chhabra M, Singh P, Bhattacharya D, Venkatesh S. Dominance shift of DENV-1 towards reemergence and co-dominant circulation of DENV-2 & DENV-3 during post-monsoon period of 2012 in Delhi, India. J Virol Retrovirol. 2014;1(1):104.
7. Islam A, Abdullah M, Tazeen A, Afreen N, Deeba F, Naqvi IH, et al. Detection of all the four serotypes of dengue virus in New Delhi, India during post monsoon season of 2015. Indian J Health Sci Care. 2016;3(3):24-9.
8. Vikram K, Nagpal BN, Pande V, Srivastava A, Saxena R, Anvikar A, et al. An epidemiological study of dengue in Delhi, India Acta Trop. 2016;153:21-7.

9. Souza SD, Bonon SH, Costa SC, Rossi CL. Evaluation of an in-house specific immunoglobulin G (IgG) avidity ELISA for distinguishing recent primary from long-term human cytomegalovirus (HCMV) infection. Rev Inst Med Trop Sao Paulo. 2003;45(6):323-6.
10. Parveen N, Broor S, Abdullah M, Hisamuddin M, Tazeen A, Asif N, et al. Resurgence of dengue-like and chikungunya-like illness in New Delhi, India during 2016. Indian J Health Sci Care. 2016;3(2):61-5.
11. Anita C, Suresh K, Neha S, Sonia M. Dengue outbreak in Delhi in 2009: study of laboratory and clinical parameters. J Commun Dis. 2012;44(3):163-8.
12. Abhishek KS, Chakravarti A. Laboratory evaluation of the SD Bioline- Dengue Duo rapid immunodiagnostic test kit for detection of NS-1 antigen and IgM antibody during dengue outbreak in Delhi. Int J Curr Res. 2018;10(05):69567-9.
13. Dar L, Gupta E, Narang P, Broor S. Cocirculation of dengue serotypes, Delhi, India, 2003. Emerg Infect Dis. 2006;12(2):352.
14. Fiorentino DF, Bond MW, Mosmann TR. Two types of mouse T helper cell. IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. J Exp Med. 1989;170(6):2081-95.
15. Abhishek KS, Chakravarti A, Baveja CP, Kumar N, Siddiqui O, Kumar S. Association of interleukin-2,-4 and-10 with dengue severity. Indian J Pathol Microbio. 2017;60(1):66.
16. de Souza VA, Fernandes S, Araújo ES, Tateno AF, Oliveira OM, Oliveira RR, et al. Use of an immunoglobulin G avidity test to discriminate between primary and secondary dengue virus infections. J Clin Microbiol. 2004;42(4):1782-4.

Cite this article as: Kumar AS, Siddiqui O, Chakravarti A. Increasing number of secondary dengue cases: a concern. Int J Res Med Sci 2019;7:1558-61.