



Research Article

Volume 4 Issue 2 – February 2018
DOI: 10.19080/APBIJ.2017.04.555632

Anatomy Physiol Biochem Int J

Copyright © All rights are reserved by Sudipta Poddar

Detection of NS1 Antigen in Serologically Confirmed Dengue Cases at least after 5 Days of Infection



Sudipta Poddar* and Amiya Kumar Hati

Department of Medical Physiology, Lincoln University College, Malaysia

Submission: January 01, 2017; Published: February 09, 2018

*Corresponding author: Sudipta Poddar, Department of Medical Physiology, Lincoln University College, 47301, Petaling Jaya, Malaysia;

Email: drsudiptapoddar@gmail.com; drspoddar@yahoo.com

Abstract

Objectives: The objective of this paper is to detect the NS1 antigen beyond 5 days of infection in serologically confirmed dengue cases in whom both NS1 antigen and IgG and IgM antibodies were assessed.

Methods: Out of a total of 1037 suspected persons 257 i.e. 24.78% were dengue reactive in 2013 and 2014. Dengue cases were diagnosed in the laboratory from suspected patients by dengue specific IgG, IgM antibodies and NS1 antigen. NS1 antigen and IgG antibodies were monitored in 220 (194+26) suspected dengue cases where interpretation was possible to detect NS1 beyond 5 days of infection. This study of detection of NS1 antigen in serologically confirmed dengue cases at least after 5 days of infection.

Results: Among this 220 cases 86 (80+6) were confirmed dengue cases. Out of these 86 cases NS1 antigen was found beyond 5 days of infection in 15 i.e. 17.44%.

Conclusion: It would be reasonable to believe that many such cases would be found in the category I where only NS1 was tested. Hence for getting the information on NS1 antigen beyond 5 days all three serological tests (NS1 antigen, IgG and IgM antibodies) should be performed. This sort of experience will help to enrich the effective control and case management of the menace.

Keywords: Dengue; NS1; IgG; IgM; Early dengue cases; Beyond 5 days; Different categories

Introduction

Dengue is the most rapidly spreading mosquito-borne arbovirus viral disease in the world. Dengue causes socioeconomic and disease burden on many tropical and subtropical regions of the world [1,2]. Dengue, infects approximately 390 million people per year. Recent disease distribution model estimated that there were 96 million apparent dengue virus infections globally in 2010 and that Asian countries, with 67 million apparent infections, bore a disproportionate infectious burden (70%) [3]. There are four distinct dengue virus serotypes, DENV-1, DENV-2, DENV-3, and DENV-4. Each of the four serotypes has been individually found to be responsible for dengue epidemics and associated with more severe dengue [4, 5].

In serological diagnosis of dengue NS1 antigen is playing a pivotal role to diagnose the disease in its early days. Many physicians depend only on NS1 antigen test for early diagnosis of dengue. NS1 produces a very strong humoral response.

NS1 antigen is detected for diagnosing dengue cases from 1st day onwards of the appearance of disease symptoms. NS1 antigen detection may have higher sensitivity during the first 5 days after the onset of symptoms thereafter NS1 antigen decreases gradually and antibody detection tests have higher

sensitivity after day 5 of the disease following onset [6]. According to WHO in the patients serum NS1 antigen is usually found circulating from the first day after the onset of fever up to the 5th day and it may be extended up to 9 days after the onset of infection in some cases [7]. The circulation of NS1 antigen from the 1st day in the patient serum after the onset of fever up to day 9 has been reported by other workers [8]. We want to study the presence of NS1 antigen in some of our serologically confirmed dengue cases beyond five days of illness during 2013 & 2014 in which monitoring of NS1 antigen beyond 5 days was positive.

Materials & Methods

Study area

Kolkata is one of the largest cities of eastern India and the capital of West Bengal. It has a land area of 185 km² and a population of about 4.496 million (2011 census). The investigative studies were conducted in the Gautam Laboratories imaging and Research Centre (NABL i.e. National Accredited Biological Laboratory) located in the thickly populated dengue endemic central part of the city of Kolkata. The study period extended from 15 January 2013 to 31st December 2014. Dengue suspected patients were referred to this pathological laboratory for confirmatory diagnosis of dengue infection.

Data collection

The patients were residence primarily of the study area; the male and female patients were of different age groups. The time of collection of serum to detect NS1 antigen is 1 to 6 days after the onset of symptoms [7]. The NS1 antigen circulates from the 1st day after the onset of fever up to day 9 [7,9]. Serological tests requested by the physicians for confirmatory diagnosis of dengue were dengue specific NS1 antigen and IgM and IgG antibodies either alone or in various combinations. According to the physicians' demand, the following categories were obtained.

Category I	Only NS1 antigen
Category II	NS1 antigen and IgM & IgG antibodies
Category III	Only IgM & IgG antibodies
Category IV	NS1 antigen & IgM antibodies
Category V	Only IgM antibodies

On very rare occasions physicians wanted to investigate NS1 antigen and IgG antibodies only (category VI) or even IgG antibodies only (category VII) When the physicians wanted to investigate IgM alone (category V) or only IgM & IgG antibodies (category III), blood was drawn from the suspected patient 5 days after the onset of fever. To test other categories, this discrimination was not followed. In the study 257 serologically confirmed dengue cases have been obtained out of which 80 cases in the category II (NS1+IgM+IgG) and 6 cases in the category IV (NS1+ IgM), totally 86 cases have been monitored. The aim is to find out the presence of NS1 antigen in the serum of the patients at least beyond five days after the onset of fever.

Table 1:

Months	Categorization																	Total
	NS1		NS1+IgM+IgG						IgM+IgG				NS1+IgM			IgM		
	E	ED	ND	O	EP	LP	ES	LS	ND	O	LP	LS	ND	ED	LD	ND	LD	
Jan	10	0	3						6							5		24/0
Feb	8	0	4					1	2	1	3		2			4		25/4
Mar	7	0	2			1				2						1		13/1
Apr	11	0	1			1			2								1	16/2
May	7	0							5	1		2			1			4-0ct
Jun	7	0							3	2						1		13/0
Jul	11	0	6	6			1			2						2		28/1
Aug	38	8	2	2					4	4	2		4			1		65/10
Sep	60	20	6	9	4	5	8	3	6	6	5	1	1		1	6	2	143/49
Oct	99	39	20	9	4	6	13	1	24	8	12	2	4		1	6	2	250/80
Nov	161	41	21	9	11	5	4	5	26	10	7	8	7	1	1	4	3	324/86
Dec	68	9	14		3	2	2		12		2		2	1		3	2	120/21
Total	487	117	79	35	22	20	28	10	90	36	31	13	20	2	4	33	10	1037/257
Gr. Total	604- 58.24%		194- 18.71%						170- 16.39%				26- 0.025%			43- 0.04%		
& %	19.37%		41.24%						25.88%				23.08%			23.26%		

Biochemical analysis

The serum samples were tested in the Laboratory for the presence or absence of dengue specific IgG, IgM antibodies and NSI, the ELISA test was performed using Bio-Rad Kits [8]. The instructions of the manufacturer were minutely followed.

Statistical analysis

Data analysis was performed using Statistical Package for Social Science (SPSS) 21.0 (SPSS Inc., Chicago, USA).

Results

In the study altogether 824 (604+194+26) NS1 related tests were performed, of which 139 (117+22) persons were NS1 reactive, 117 in Category I and 22 in the Category II (EP i.e. early primary). Along with these NS1 reactive cases another 15 NS1 reactive cases were detected 13 out of 20 in LP i.e. late primary stage, 1 in Category II (out of 10) in LS i.e. late secondary and 1 in Category IV (out of 4) in LD i.e. late dengue (Table 1). In these 15 NS1 reactive dengue confirmed patients NS1 antigen became positive at least after 5 days of dengue infection. So in late primary 20 cases 13 are NS1 and IgM positive, among 10 late secondary cases only one case is NS1, IgG, IgM positive and in only one case IgM and NS1 are positive which is late dengue. (Table 1). Table 2 presents detail serological results along with categorization of 15 confirmed dengue cases in whom NS1 antigen has been detected beyond 5 days of infection. Of those patients 8 are males. Age of the patients varies from 7 to 65 years.

E: Suspected cases in whom NS1 not detected; LP: Late Primary; ED: Possibly Early Dengue; ES: Early Secondary; ND: Not Dengue; LS: Late Secondary; O: Old Dengue Cases; LD: Late Dengue; EP: Early Primary

Table 2: Dengue cases with NS1 antigen present at least after 5 days of infection in different categories of dengue patients in 2013-2014.

NS1 Antigen Positive after 5 days						
No.	Age	Sex	Serological Results			Category of Patients
1	24	M	NS1 R	IgM R	IgG NR	Late primary
2	10	F	NS1 R	IgM R	x	Late dengue
3	23	M	NS1 R	IgM R	IgG NR	Late primary
4	65	F	NS1 R	IgM R	IgG NR	Late primary
5	8	F	NS1 R	IgM R	IgG NR	Late primary
6	18	F	NS1 R	IgM R	IgG NR	Late primary
7	39	F	NS1 R	IgM R	IgG NR	Late primary
8	20	M	NS1 R	IgM R	IgG NR	Late primary
9	18	M	NS1 R	IgM R	IgG NR	Late primary
10	12	F	NS1 R	IgM R	IgG NR	Late primary
11	35	M	NS1 R	IgM R	IgG NR	Late primary
12	20	M	NS1 R	IgM R	IgG NR	Late primary
13	37	M	NS1 R	IgM R	IgG NR	Late primary
14	29	M	NS1 R	IgM R	IgG NR	Late primary
15	7	F	NS1 R	IgM R	IgG R	Late secondary
Total		15 cases				

So far as categorization of those 15 dengue infected patients is concerned,

- a) 13 cases are NS1 and IgM reactive (Late Primary)
- b) 1 case is NS1, IgG and IgM reactive (Late Secondary)
- c) 1 case is IgM, NS1 reactive (Late Dengue)

So, most of the cases (13 out of 15) are late primary dengue cases.

Discussion

NS1 antigen was found beyond 5 days in 17.44% (15/86). This phenomenon of detection of NS1 beyond 5 days of initiation of infection indicated that all early cases of dengue were not actually early cases. In Category I in 2013 and 2014 altogether 117 NS1 reactive cases were detected and all these cases were regarded as early dengue cases. It was shown above that in 15 NS1 reactive cases NS1 was present beyond 5 days of infection. So, all early dengue cases might not actually be the early cases. In at least some of them NS1 antigen might be reasonably found after 5 days of initiation of infection. So, NS1 test alone cannot put forward the exact information, whether the dengue infection is an early infection or not. To get the accurate information, all the three tests (NS1, IgM and IgG) should be performed simultaneously. NS1 antigen for early diagnosis and serotyping of dengue virus infections were carried out in China, when NS1 was detectable in blood circulation from 1st day upto day 18 after onset of symptoms [10].

NS1 antigen as an early diagnostic marker in dengue was reported from India [11]. NS1 antigen test was able to detect dengue virus infection from days 1 to 8 in 44% of samples [12]. According to another study the sensitivity of the NS1, IgM ELISA was higher when compared with RT PCR and therefore it was recommended to use for early diagnosis [13]. NS1 antigen to diagnose early dengue infection was also reported in another study in India [14]. In those studies mentioned above the proportion of dengue cases positive with NS1 antigen beyond 5 days of infection was actually not available. The purpose of the study was to find out the proportion of dengue cases positive with NS1 antigen after 5 days of onset of infection. Presence of dengue specific NS1 antigen signifies early infection. This will facilitate monitoring of the patients from the beginning which is very important, especially in dengue infection.

Based on this phenomenon 58.24% of the physicians in the present study (2013-14) depended only on detection of NS1 antigen alone. But the data indicated that at least in 17.44% (15/86 of patients) suffering from dengue NS1 antigen was detected beyond 5 days of infection. It is also necessary to find out from the beginning, whether the patient is suffering from primary or secondary dengue infection. Dependence only on NS1 antigen may provide incorrect information so far as early dengue infection is concerned. So it is recommended to perform three serological tests simultaneously for better monitoring of dengue patients which will provide all necessary information mentioned above.

Acknowledgement

The authors are thankful to Prof. Datuk Dr. Abdul Gani Bin Mohammed Din, Deputy Vice Chancellor (Academic), Lincoln University College and Prof. Dr. Amiya Bhaumik, CEO and Vice Chancellor for giving necessary permission to proceed the work and also to the Directors of Gautam Laboratories Imaging and Research Centre, India for various helps.

References

1. Guzmán MG, Kourí G (2002) Dengue: an update. *Lancet Infect Dis* 2(1): 33-42.
2. Gubler DJ (2011) Dengue, Urbanization and Globalization: The Unholy Trinity of the 21st Century. *Trop Med Health* 39(4 Suppl): 3-11.
3. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, et al. (2013) The global distribution and burden of dengue. *Nature* 496(7446): 504-507.
4. WHO Regional Office for South-East Asia (2011) Comprehensive Guidelines for Prevention and Control of Dengue and Dengue Haemorrhagic Fever, Revised and Expanded Edition . World Health Organisation New Delhi South East Asia Regional Office New Delhi, India pp. 196.
5. Gibbons RV, Vaughn DW (2002) Dengue: an escalating problem. *BMJ* 324(7353): 1563-1566.
6. Dutta AK, Biswas A, Baruah K, Dhariwal AC (2011) National guidelines for diagnoses and management of dengue fever/dengue haemorrhagic fever and shock syndrome. *J Indian Med Assoc* 109(1): 30-35.
7. TDR Dengue guide line for diagnosis, treatment, prevention and control WHO (2009) pp. 145,146.
8. Bio-Rad (2018) 3 boulevard Raymond Poincare, 92430 names la. Coquette, France.
9. Alcon S, Talarmin A, Debruyne M, Falconar A, Deubel V, et al. (2002) Enzyme-linked Immunosorbent assay specific to dengue virus type 1 Nonstructural protein NS1 reveals circulation of the antigen in the blood during the acute phase of disease in patients experiencing primary or secondary infection. *J Clin Microbiol* 40(2): 376-381.
10. Hua Xu, Biao Di, Yu-xian Pan, Li-wen Qiu, Ya-di Wang, et al. (2006) Serotype 1-specific monoclonal antibody-based antigen capture immunoassay for detection of circulating Nonstructural protein NS1: Implications for early diagnosis and serotyping of dengue virus infections. *J Clin Microbiol* 44(8): 2872-2878.
11. Singh MP, Majumdar M, Singh G, Goyal K, Preet K (2010) NS1 antigen as an early diagnostic marker in dengue: report from India. *Diagn Microbiol Infect Dis* 68(1): 50-54.
12. Kassim FM, Izati MN, TgRogayah TA, Apandi YM, Saat Z (2011) Use of dengue NS1 antigen for early diagnosis of dengue virus infection. *Southeast Asian J Trop Med Public Health* 42(3): 562-569.
13. Gowri Sankar S, Dhananjeyan KJ, Paramasivan R, Thenmozhi V, Tyagi BK, et al. (2012) Evaluation and use of NS1 IgM antibody detection for acute dengue virus diagnosis: report from an outbreak investigation. *Clin Microbiol Infect* 18(1): E8-E10.
14. Bhattacharyya N, Mukherjee H, Naskar R, Talukdar S, Das G, et al. (2014) Serological diagnosis of dengue laboratory practice in Kolkata. *Ind Jour Med Micro Biol* 32(3): 277-280.



This work is licensed under Creative Commons Attribution 4.0 License
DOI: [10.19080/APBIJ.2017.04.555632](https://doi.org/10.19080/APBIJ.2017.04.555632)

Your next submission with Juniper Publishers will reach you the below assets

- Quality Editorial service
- Swift Peer Review
- Reprints availability
- E-prints Service
- Manuscript Podcast for convenient understanding
- Global attainment for your research
- Manuscript accessibility in different formats (Pdf, E-pub, Full Text, Audio)
- Unceasing customer service

Track the below URL for one-step submission

<https://juniperpublishers.com/online-submission.php>