ORIGINAL RESEARCH PAPER
Volume - 13 | Issue - 03 | March - 2024 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

CORRELATION OF DENGUE VIRAL LOAD WITH DENGUE DISEASE SEVERITY AND SEROLOGY AMONG CHILDREN AND ADULTS ATTENDING A TERTIARY CARE HOSPITAL IN MUMBAI, INDIA

Clinical Microbiology

Dr Sandhya Sawant Associate Professor (Additional), Department of Microbiology, TNMC & BYL Nair Charitable hospital, Mumbai.

Dr Seema Gaikwad Ex Resident, Department of Microbiology, TNMC & BYL Nair Charitable hospital, Mumbai.

Dr Jayanthi Shastri Ex Professor & Head, Department of Microbiology, TNMC & BYL Nair Charitable hospital, Mumbai.

ABSTRACT

Introduction - Dengue fever is spreading rapidly around the world and is caused by any of four dengue virus serotypes (DENV-1, 2, 3, and 4) and is a major public health problem. During an outbreak, the number of seriously ill people visiting hospitals can overwhelm public health systems in many urban centres. Current information about why some people infected with dengue develop severe illness and others remain asymptomatic or have mild illness is not yet understood. **Material And Methods -** A Prospective laboratory study was conducted over a period of 6 months (n = 200) in year 2015 in the Department of Microbiology and Molecular diagnostic Reference laboratory associated with tertiary care hospital. Approval of Institutional Ethics Committee was taken prior to beginning of the study. **Results -** Of 200 clinically suspected Dengue cases, 119 (82.4 %) were positive for Dengue virus confirmed by RT-PCR. Of the 119 laboratory confirmed cases, 98 (82.4 %) presented as Dengue Fever (DF), 17 (14.3%) presented as Dengue Haemorrhagic Fever (DHF) and 4 (3.4%) presented as Dengue shock syndrome (DSS). The distribution of viral load differed significantly in dengue fever (P-value<0.01) and was found in the reference range of Log 10^4 to Log 10^5. The viral load was found less in samples positive for IgM antibodies. **Discussion And Conclusion -** Correlation of viral load with clinical and serological data can provide important information on the aetiology of various dengue-related syndromes such as dengue haemorrhagic fever and dengue shock syndrome. This is because multiple factors, including interactions between immune characteristics, are involved in disease progression and complications.

KEYWORDS

Dengue viral load, serology, disease severity

INTRODUCTION

Dengue is a rapidly spreading mosquito borne disease in the world caused by any of four serotypes of dengue virus (DENV-1, 2, 3 and 4), and is a major public health concern recently. During outbreaks, the number of people reporting to clinics with severe disease can overwhelm the public health systems of many urban centres.[1] Clinical manifestations include a wide range of symptoms and signs, from subclinical disease to mild undifferentiated fever, to serious and potentially fatal complications like dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS). Severe dengue cases are increasing in endemic areas of Southeast Asia, Central and South America, and other subtropical regions. The most commonly affected group is children.[2,3] Pathogenesis is attributed to multiple factors, including interactions between viral traits, immune traits, and the genetic background of the host. In endemic areas infection with different serotypes is believed to be one of the most significant factor affecting dengue severity.[4,5] Several molecular markers such as dengue virus load, serotype, dengue NS1 antigen (non-structural protein 1), immunoglobulin M (IgM), immunoglobulin G (IgG) antibodies (anti-dengue IgM and IgG) were measured to determine the severity as well as for diagnosis of dengue fever. Dengue serotype and viral load are two important factors that regulate the outcome of dengue infection. [6,7] Dengue NS1 antigenemia is detected within 24 hours and persists for 9 days after onset. Simultaneous testing of dengue NS1 and anti-dengue IgM/IgG allows diagnosis of both early and late dengue. [8] These serological and molecular markers are used not only to confirm the diagnosis, but also to classify the severity of the disease or the increased risk of progressing to more serious forms of dengue infection. In several studies, these markers have been studied either singly or combined in different geographical locations. [9,10]

Current information about why some people infected with dengue develop severe illness and others remain asymptomatic or have mild illness has been ill understood. [6]

Therefore this study was conducted to understand the relationship between plasma viral load with severity of dengue and interrelationship of all serological markers.

MATERIAL AND METHODS

A Prospective laboratory study was conducted over a period of 6 months from June to December in the year 2015 in the Department of Microbiology and Molecular diagnostic Reference laboratory associated with tertiary care hospital. Approval of Institutional Ethics Committee was taken prior to beginning of the study. Before proceeding with any kind of test, informed consent was obtained from the subjects. In case of paediatric patients, assent from the parents was obtained.

Two hundred clinically suspected cases of dengue presenting with fever from 1-9 days in the age group of 1-80 years along with symptoms like headache, myalgia, retro-orbital pain, rash, haemorrhagic manifestations were included in the study.

Approximately 3-5 ml of blood sample was collected aseptically from clinically suspected dengue fever patients after written informed consent and /or assent in plain and EDTA vacutainer. Additionally, relevant demographic, clinical and investigational information was collected from patient's record file. Detailed clinical history of the patient with relevant records was recorded in case record form.

Collected samples were centrifuged at 2500 rpm for 10 minutes to obtain serum and plasma. Serum samples were tested for detection of NS1 antigen using rapid immunochromatographic tests (Aspen Lab Pvt Ltd) and antidengue IgM and IgG antibodies. (SD Biostandard) The results of rapid tests were further confirmed by ELISA (NS1 ELISA – CTK Biotech, IgM ELISA – J Mitra.) Real Time RT PCR was carried out on all samples for laboratory confirmation of dengue cases.

Table 1. Primers And 5'nuclease Probes

Virus Gene Bank Accession no	Sense primer, antisense primer, Probe [Sequence Position]	Genomic Target Region	Amplicion length (bp)
DENV (M87512)	DenS (GGATAGACCAGAGATCC TGCTGT [10615–10636]) DenAs (CATTCCATTTTCTGGCGT TCplusCAATCCATCTTGC GGCGCTC, 1:1 [10694–10675]) DenP (CAGCATCATTCCAGGCAC AG [10656–10675])	3'- noncoding region	79

Real Time RT- PCR

RNA extraction was done using automated MagNA Pure 96, (Roche System) for viral RNA extraction from samples. The extracted nucleic acid elute from patients sample was then subjected to Real Time PCR and the following probes and primers were used. Samples were subjected to PCR conditions as per the manufacturer's instructions. PCR products were detected in real time on a Light Cycler instrument by using 5'-nuclease technology. PCR results were obtained within 90 minutes (55 cycles and melting curve).

Quantification of Viral load using RT PCR was done on all laboratory confirmed PCR positive samples. Viral load was detected in logarithmic multiples as copies/µl of plasma sample. Plasmid viral DNA used had concentration of Log 10^7 in lyophilised form. A standard curve was prepared. This when used to quantitate after a standard curve was produced could detect viral load of Log 10^7 (E7) maximum to a lowest of Log 10^1 (E1) copies/µl. To elaborate,1.34 Log 10^3 was the lowest detected viral load ,while 2.87 Log 10^7 copies/µl was the highest detected viral load in our PCR positive samples.

Table 2. Reference Chart For Viral Load Values

Viral load in copies/ul	Reference Viral Load
1.34 to 4.99 Log 10^3	E3
1.12 to 9.23 Log 10^4	E4
1.03 to 6.66 Log 10^5	E5
2.31 to 3.50 Log 10^6	E6
2.38 to 2.87 Log 10^7	E7

Statistical Methods

The entire data was entered and cleaned in MS Excel before its statistical analysis. All the results are shown in tabular and graphical format, wherever necessary to visualize the statistically significant difference more clearly. The p-values less than 0.05 were considered to be statistically significant. All the hypotheses were formulated using two tailed alternatives against each null hypothesis (hypothesis of no difference). The entire data was analysed statistically using Statistical Package for Social Sciences (SPSS ver 11.5, Inc. Chicago, USA) for MS Windows.

RESULTS

Of 200 clinically suspected Dengue cases, 119 (82.4 %) were positive for Dengue virus confirmed by RT-PCR. Of the 119 laboratory confirmed cases, 98 (82.4 %) presented as Dengue Fever (DF), 17 (14.3%) presented as Dengue Haemorrhagic Fever (DHF) and 4 (3.4%) presented as Dengue shock syndrome (DSS).

The common clinical presentations among the patients were Fever in 98.5 % and Chills 100 %. Median age of the study population was 22.7 years and sex ratio (Male: Female) was 1.4:1. Out of 200 samples subjected to different test, RT-PCR was positive in 119 (59.5%) and negative in 81 (40.5%) for Dengue virus. Serological response in the form of NS1, IgM and IgG antibodies was seen in 119 RT PCR positive patients. NS1 antigenemia was found in 86 (72.3%) patients and IgM/IgG antibody response with or without NS1 antigenemia was found in 33 (27.7%) patients.

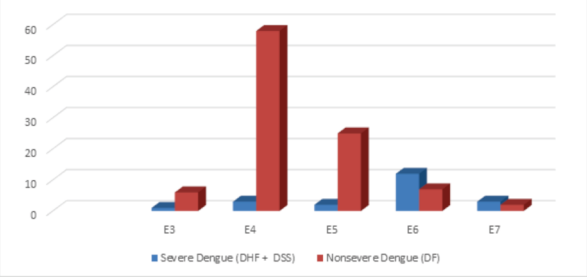

Figure 1. Association Between Plasma Viral Load And Disease Severity

Table 3. The Distribution Of Viral Load According To Clinical Manifestation Dengue Cases (n= 119)

Viral Load	Dengue Fever		Dengue Haemorrhagic Fever		Dengue Shock Symdrome	
	No of Cases	% of Cases	No of Cases	% of Cases	No of Cases	% of Cases
E3	6	6.12	0	0	1	25
E4	58	59.2	2	11.8	1	25
E5	25	25.51	2	11.8	0	0
E6	7	7.14	10	58.8	2	50
E7	2	2.04	3	17.6	0	0
Total	98	100	17	100	4	100

The distribution of viral load differed significantly in dengue fever (P-value<0.01). Maximum patients had viral load of Log 10^4 whereas viral load was found high in the range of Log 10^6 to Log 10^7 in majority of patients with dengue haemorrhagic fever (Pvalue<0.01).

Table 4. The Distribution Of Viral Load And Serology

		Serology					
Clinical Syndrome	Viral Load	NS1 POS	NS1+IgM POS	NS1+IgM +IgG POS	IgM POS	IgM+IgG POS	Total
DF	E3	5	1	0	0	0	6
n =98	E4	44	4	4	4	2	58
	E5	17	3	0	3	2	25
	E6	5	1	1	0	0	7
	E7	1	1	0	0	0	2
DHF	E3	0	0	0	0	0	0
n =17	E4	0	2	0	0	0	2
	E5	0	2	0	0	0	2
	E6	8	2	0	0	0	10
	E7	2	1	0	0	0	3
DSS	E3	0	0	1	0	0	1
n =4	E4	0	0	0	1	0	1
	E5	0	0	0	0	0	0
	E6	2	0	0	0	0	2
	E7	0	0	0	0	0	0

Table 5. The Distribution Of Viral Load According To Clinical Manifestation In Various Age Groups Of Dengue Cases (n= 119).

Clinical Syndrome	Age Group in years						Total
	Viral Load	1-10	11-20	21-30	31-40	41-50	>51	
DF	E3	1	1	4	0	0	0	6
	E4	10	8	17	16	6	1	58
	E5	19	4	1	1	0	0	25
	E6	5	0	2	0	0	0	7
	E7	1	0	1	0	0	0	2
DHF	E3	0	0	0	0	0	0	0
	E4	2	0	0	0	0	0	2
	E5	0	0	2	0	0	0	2
	E6	1	5	6	2	0	0	14
	E7	0	2	1	0	0	0	3
DSS	E3	1	0	0	0	0	0	1
	E4	0	0	0	0	1	0	1
	E5	0	0	0	0	0	0	0
	E6	0	0	2	0	0	0	2
	E7	0	0	0	0	0	0	0

DISCUSSION

The study involves use of quantitative RT-PCR method, which is more sensitive and convenient than virus isolation, to decipher the relationship between plasma dengue viral load and disease severity during the course of infection.

The distribution of viral load in different clinical manifestations like DF, DHF and DSS was studied. It was found that the distribution of viral load differed significantly (p<0.01) between Dengue fever (DF) & DHF. Majority of patients had viral load of Log 10^4 in dengue fever whereas viral load was found high in the range of Log 10^6 to Log 10^7 in patients with dengue haemorrhagic fever (DHF). **Table 3**

It has been proposed that dengue virus replication in humans occurs mainly in mononuclear phagocytes and that the pathogenesis of DHF may be related to the number of these cells infected with dengue virus. Common hypothesis would be that patients with severe and fatal disease should have more infected mononuclear cells and presumably more virus.[11] This would imply that patients with severe disease should have higher viremias than those with milder disease, provided virus is released from the cells.[12] Gubler et al have shown this correlation. Our study results also showed significantly high viral load values among patients with mild and severe disease. Our study shows viral load in the range of Log 10^6 to Log 10^7 in most of DHF and DSS patients. Plasma viral load was found in the range of Log 10^4 in DF patients. In comparison, two DSS patients had low viral load. Earlier studies show that the blood of patients with severe dengue shock syndrome may be

virologically sterile because of the antigen-antibody complexing that occurs.[13] Murgue et al reported that the magnitude of viraemia and severity of disease had a correlation based on haemagglutination inhibition assay. They also looked for the duration of viremia in each clinical presentation, which was not included in our study. The small sample size of severe dengue cases may be one of the factor which could not conclusively prove the correlation between the viral load and severity.[14]

In this study, we have made an attempt to find association of plasma viral load with disease severity . **Figure 1.** DHF and DSS was considered as severe, while DF was considered as mild form of disease. Out of total 21 severe dengue patients, 10 patients had viral load of Log 10^6. Among nonsevere dengue patients, maximum patients had viral load of Log 10^4 and Log 10^5.It was found that viral load was different in different patients. Our study results conform with Gubler et al who noted a similar finding,[13] However,Murgue et al stated that there is a correlation between disease severity and magnitude of viral load, using haemagglutination assay to detect viral load.[14] Two of our patients succumbed, due to complications and had a viral load of Log 10^4 and Log 10^5. Association of dengue viral load and disease severity are different in various studies as multiple factors are involved in disease progression and complications. [13,14]

The distribution of viral load and serology was also studied in detail. **Table 4** We found that NS1 antigenemia was seen predominantly in patients having high viral load which was also found more in severe dengue patients. The maximum number of nonsevere dengue patients had viral load in the reference range of Log 10^4. Ten of the severe dengue patients had high viral load in the reference range of Log 10^6. Our study showed high viral load in the presence of dengue NS1. NS1 antigenemia is associated with high viral load and is independent of immune status. [15] Samples positive for anti dengue IgM antibodies had viral load in the reference range of Log $10^4$and Log 10^5 suggesting IgM as a strong modulator of viraemia. Inverse corelation between IgM seropositivity and viral load has been observed in various studies. This may happen due to clearance of virus by IgM antibodies.[16,17]

The viral load distribution in various age groups was also studied. The distribution of viral load in various age groups was not found to be statistically significant in our study patients ($p<0.05$). **Table 5** We concluded that there was no significant variation in viral load among patients presenting with different clinical manifestation, in different age groups. These results, though based on a small sample size examined for each value of viral load indicate that DF and DHF patients have different patterns of viremia during the course in a particular age group. We observed that maximum patients with DF had a viral load of 10^5 copies/ul, most of them were from pediatric age group. It is well documented that paediatric patients are more susceptible to severe disease because of low immunity. Children loose a lot of water and fluids after a dengue attack. So a second attack in children can be fatal. [18] Wang et al reported that DF and DHF have different patterns of viral load.[19] While many studies have studied viral load distribution among paediatric population. Manjith Narayanan et al Thomas et al who studied paediatric population for disease severity noted that high viral load was associated with DENV -2 infection and with DHF/DSS in patients presenting on 3rd day of illness. [1, 20] Severe effects of Dengue resulted from the combined effects of deng-2 and secondary dengue infection. We propose that studies should be carried out to see the correlation of serotypes with viral load and disease progression.

Despite limitations in our study, our data demonstrate individual differences in dengue viral load patterns among different clinical presentations, thereby indicating that host processes are critical in regulating viral dynamics. Due to the dynamic nature, further analysis of additional cases should be investigated for infection with accompanying serotypes and disease severity. There is an urgent need to study the impact of dengue virus serotypes on viral loads with different clinical outcome.

CONCLUSION

Correlation of viral load with clinical and serological data can provide important information on the etiology of various dengue-related syndromes such as dengue hemorrhagic fever and dengue shock syndrome. We found that the formation of antibodies reduced the viral load due to the formation of immune complexes. Several factors are involved in disease progression and complications, including viral traits, immune traits, and interactions between host genetic background and high endemic disease.

REFERENCES

1. Laurent Thomas, Olivier Verlaeten, André Cabié, StéphaneKaidomar, Victor Moravie, Jenny Martial, FatihaNajioullah, Yves Plumelle, Christiane Fonteau, Philippe Dussart, and Raymond Césaire Influence of the Dengue Serotype, Previous Dengue Infection, and Plasma Viral Load on Clinical Presentation and Outcome During a Dengue-2 and Dengue-4 Co-Epidemic Am. J. Trop. Med. Hyg., 78(6), 2008, pp. 990–998
2. Whitehead SS, Blaney JE, Durbin AP, Murphy BR. Prospects for a dengue virus vaccine. *Nat Rev Microbiol*2007; *5:* 518–28.
3. Gubler DJ, Clark CG 'Dengue/Dengue hemorrhagic fever: The emergence of global health problem'.Emerg Infect Dis.1995;1(2):55-57.
4. Gubler DJ, 1998. Dengue and dengue hemorrhagic fever. ClinMicrobiol Rev 11: 480–496.
5. Guzman MG, Kouri G, 2002. Dengue: an update. Lancet Infect Dis 2: 33–42.
6. Guzman MG, Alvarez M, Halstead SB. Secondary infection as a risk factor for dengue hemorrhagic fever/dengue shock syndrome: An historical perspective and role of antibody-dependent enhancement of infection. Arch Virol 2013; 158 : 1445-59.
7. Vaughn DW, Green S, Kalayanarooj S, Innis BL, Nimmannitya S, Suntayakorn S, et al. Dengue viremia titer, antibody response pattern, and virus serotype correlate with disease severity. J Infect Dis 2000; 181 : 2-9.
8. Tahmina Akther , A.K.M. Muraduzzaman, S. Monira Parvin, Shahina Tabssum & Saif Ullah Munshi, Molecular & serological study of dengue virus-infected patients attending a tertiary hospital of Dhaka city, Bangladesh (2013 to 2016), Indian J Med Res 150, July 2019, pp 96-100
9. Thomas L, Najioullah F, Verlaeten O, Martial J, Brichler S, Kaidomar S, et al. Relationship between nonstructural protein 1 detection and plasma virus load in dengue patients. Am J Trop Med Hyg 2010; 83 : 696-9.
10. De La Cruz Hernández SI, Flores-Aguilar H, González-Mateos S, López-Martínez I, Ortiz-Navarrete V, Ludert JE, et al. Viral load in patients infected with dengue is modulated by the presence of anti-dengue IgM antibodies. J Clin Virol 2013; 58 : 258-61.
11. Wei-Kung Wang,,1 Day-Yu Chao, Chuan-Liang Kao, Han-Chung Wu, Yung-Ching Liu, Chien-Ming Li, Shih-Chung Lin, Shih-Ting Ho, Jyh-Hsiung Huang, and Chwan-Chuen King, High Levels of Plasma Dengue Viral Load during Defervescence in Patients with Dengue Hemorrhagic Fever: Implications for Pathogenesis Virology 305, 330–338 (2003)
12. Wei-Kung Wang,1,3 Hui-Ling Chen,1 Chao-Fu Yang,1 Szu-Chia Hsieh,1 Chung-Chou Juan,5 Shu-Mei Chang,5 Cheng-Ching Yu,6 Li-Hui Lin,6 Jyh-Hsiung Huang,4 and Chwan-Chuen King2 Slower Rates of Clearance of Viral Load and VirusContaining Immune Complexes in Patients with Dengue Hemorrhagic Fever CID 2006:43 (15 October) 1023
13. Gubler, D. J., Suharyono, W., Tan, R., Abidin, M., and Sie, A. (1981). Viremia in patients with naturally acquired dengue infection. Bull. WHO 59, 623–630.
14. MurgueB,RocheC,Chungue E ,et al.Prospective study of the duration and magnitude of viremia in children hospitalised During the 1996-1997 Dengue -2 Outbreak in French Ploynesia.J Med Virol 200:60:432-438
15. Laurent Thomas, Fatiha Najioullah, Olivier Verlaeten, Jenny Martial, Segolene Brichler, Stephane Kaidomar, Victor Moravie, Andre Cabie and Raymond Cesaire, Relationship between nonstructural protein 1 detection and plasma virus load in dengue patients, Am J Trop Med Hyg, 2010 Sept; 83(3): 696-699.
16. Cologna R, Armstrong PM, Rico-Hesse R. Selection for virulent dengue viruses occurs in humans and mosquitoes. J Virol 2005; 79 : 853-9.
17. Felix AC, Romano CM, Centrone Cde C, Rodrigues CL, Villas-Boas L, Araújo ES, et al. Low sensitivity of NS1 protein tests evidenced during a dengue type 2 virus outbreak in Santos, Brazil, in 2010. Clin Vaccine Immunol 2012; 19 : 1972-6.
18. Guidelines for clinical management of dengue fever, dengue haemorrhagic fever and dengue shock syndrome. Directorate of national vector borne diseases control programme 2008.
19. Wang WK, Chao DY, Kao CL, Wu HC, Liu YC, Li CM, et al. High levels of plasma dengue load during defervescence in patients with dengue hemorrhagic fever: implications for pathogenesis. Virol 2003;305:330-8
20. Naraynan M, Aravind MA, Thilothammal N, Prema R, Sargunam CS, Ramamurty N. Dengue fever epidemic in Chennai-a study of clinical profile and outcome.; Indian Pediatr. 2002;39(11):1027-33.