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Original Article

Quantitative Estimation of Dengue NS-1 Antigen by Enzyme-Linked Immunosorbent Assay with its Clinical and Laboratory Correlation – An Observational Study

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ABSTRACT

Objectives: Dengue is a public health concern globally, which requires early and accurate diagnosis to reduce patient morbidity and mortality. This study aimed to evaluate the quantitative estimation of dengue non-structural (NS)-1 antigen by enzyme-linked immunosorbent assay (ELISA) with its clinical and laboratory correlation.

Material and Methods: This observational study was conducted in a tertiary care setting over one-and-a-half years with 280 symptomatic individuals as the study sample. Blood samples were collected, excluding patients with other diseases. Patient parameters included age, gender, symptoms, comorbidities and risk category. Quantitative ELISA was used to estimate NS1 antigen level, and statistical analysis was performed.

Results: About 86.8% and 92.1% of symptomatic individuals were positive for qualitative and quantitative ELISA NS1 tests, respectively, while rapid antigen tests showed 96.1% positive results. High mortality rates were observed in 18.9% of patients. Quantitative ELISA NS1 had significant negative ($P = 0.001$) correlations with total Leucocyte count (TLC), platelet count, pulse pressure and serum glutamate oxaloacaetic transaminase (SGOT) but a significant positive ($P = 0.024$) correlation with serum glutamic pyruvic transaminase (SGPT). Mortality rates had a significant correlation ($P = 0.040$).

Conclusion: Our findings highlight the utility of quantitative estimation of dengue NS-1 antigen by ELISA, demonstrating strong correlations with clinical and laboratory parameters. The high positivity rates observed with quantitative ELISA NS1 tests, alongside the notable mortality rates, emphasise the severity of dengue infections. Further research and clinical validation are warranted to enhance our understanding of dengue pathogenesis and refine diagnostic and management strategies to improve patient outcomes.

Keywords: Dengue non-structural 1, Quantitative enzyme-linked immunosorbent assay, Rapid antigen test, Laboratory parameters

INTRODUCTION

Dengue fever, a flavivirus infection spread by the Aedes mosquito, is the most prevalent and widely spread viral haemorrhagic fever in the world, mainly in the Americas, Pacific islands and continental Asia.[1] There are four serotypes of dengue virus that is, dengue (DEN) 1 to DEN 4. Three structural proteins are encoded by genomic RNA: C (core protein), M (membrane protein) and E (extracellular protein) (envelope protein) while non-structural (NS) 1, NS2a, NS2b, NS3, NS4a, NS4b and NS5 are the NS proteins. NS1 is a highly conserved glycoprotein that is required for virus viability but has yet to be recognised as having any biological action.[2] NS1 can be detected during the acute phase of dengue virus infections, and NS1 tests are recommended because they can be as sensitive as molecular tests within the first 0–7 days of symptoms. However, NS1 tests are not advised after day 7. A positive NS1 test result indicates dengue infection, but it does not provide serotype information, which is only required for surveillance. While NS1 can be found in whole blood or plasma, the majority of NS1 tests have been developed and evaluated on serum samples. Although combined testing with NS1 and immunoglobulin M (IgM) antibody tests can provide a diagnostic result within the first 1–7 days of illness, if both antigen and antibody tests are negative, a second convalescent-phase specimen should be obtained and tested for IgM.[3]

Dengue fever has been on the rise for decades, and the true number of cases is significantly underestimated. Globally, it is estimated that 390 million individuals are infected, with 96 million developing clinical illness,[4] producing major

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morbidity and mortality, particularly in low- and middle-income countries and posing a significant economic burden. Asia is accountable for 70% of the disease burden. Dengue fever has surged by more than eightfold in the last two decades, affecting the younger population predominantly.[5,6]

Dengue infections must be diagnosed rapidly and accurately to reduce patient morbidity and mortality. This study was conducted to aid in the quick diagnosis of this critical illness by quantitative approaches that were missed by qualitative methods for early diagnosis, disease surveillance and timely management to save lives.

Therefore, this study aimed to evaluate the quantitative estimation of dengue NS1 antigen, estimate the proportion of dengue at a tertiary care hospital, correlate the quantitative dengue NS1 with other laboratory parameters and clinical presentation, associate the quantitative levels of NS1 antigen with dengue complications and associate the quantitative levels of NS1 antigen with the mortality rates.

MATERIAL AND METHODS

An observational cross-sectional study was conducted for 18 months within a tertiary care centre, following approval from the Institutional Ethics Committee (IEC/DISS/17118). The study focussed on evaluating dengue symptoms among patients presenting at various outpatient departments and inpatient departments of the hospital. A sample of 280 symptomatic individuals underwent preliminary assessment through qualitative enzyme-linked immunosorbent assay (ELISA) before comprehensive evaluation. Blood samples were procured from suspected dengue cases subsequent to obtaining informed consent. Exclusion criteria encompassed individuals testing positive for leptospira IgM antibody, malaria or chikungunya IgM antibody. Patient-specific parameters such as age, gender, symptoms, comorbidities, total leucocyte count, platelet count and risk categorisation were recorded. A comprehensive clinical history, along with pertinent test data, was collected utilising a standardised case record form. Data collation was executed through Microsoft Excel, followed by subsequent statistical analysis including Pearson Chi-square, Shapiro–Wilk test, Spearman's rho correlation and Z-statistics.

RESULTS

During the study period, 280 symptomatic individuals over the age group two to 72 years [Table 1] with 68% males and 32% females formed our study sample. Patients mostly presented with fever (100%) chills (95%.7) and body aches (95.4) [Table 1]. Hypertension was seen in 2.1% of the patients while most of them had no associated comorbidities (96.8%) [Table 1]. Bleeding tendencies (4.3%) were the most commonly observed clinical complications in patients [Table 1]. With 98% of the patients discharged, death was observed in only 2% of the patients. For the qualitative ELISA test, 86.8% of patients were found to be positive, 92.1% of patients were positive for quantitative ELISA whereas rapid antigen test showed 96.1% positive results. All the parametric variables (TLC, platelets count, pulse pressure [mm Hg], SGOT [IU/L], SGPT [IU/L]) failed the normality test with Shapiro–Wilk test and was found to be statistically significant for all these variables ($P < 0.05$). Spearman rho correlation was performed between the parametric variables and quantitative NS1. Negative and statistically significant ($P = 0.001$) correlation was observed between quantitative NS1 and the parameters including TLC, platelet count, pulse pressure and SGOT whereas a positive and statistically significant ($P = 0.024$) correlation was found between quantitative NS1 and SGPT [Table 2]. No statistically significant association was seen between the complications and quantitative ELISA test result ($P = 0.110$) [Table 3]. A statistically significant association was seen between the mortality rate and quantitative ELISA test result ($P = 0.040$) [Table 4], whereas no statistically significant association was seen between the clinical outcome and quantitative ELISA test result ($P = 0.510$), which showed that clinical outcome was independent of the quantitative ELISA test results [Table 5].

Table 1: Distribution According to Age, Presenting Symptoms, Associated Comorbidities, and Clinical Complications

Distribution Parameters	Number (No.)	Percentage (%)
Age		
<=20 years	57	20.4
21-40 years	182	65.0
41-60 years	39	13.9
>60 years	2	0.7
Total	280	100.0
Presenting Symptoms		
Fever	280	100.0
Chills	268	95.7
Body ache	267	95.4
Headache	1	0.4
Irritability	1	0.4
Stomach-ache	1	0.4
Vomiting	1	0.4
Associated Comorbidities		
None	271	96.8
Hypertension	6	2.1
Diabetes Mellitus	4	1.4
Hypothyroidism	1	0.4
Clinical Complications		
None	253	90.4
Bleeding tendency	12	4.3
Nosebleed	8	2.9
Cold clammy extremities	2	0.7
Petechiae	2	0.7
Shock	2	0.7
Dehydration	1	0.4

Table 2: Spearman's rho Correlation Between Various Parametric Variables and Quantitative ELISA NS-1 Value

Pair	'r' Value	*P*-Value	Interpretation
Quantitative NS1 (IU/dL) and TLC	-0.607	0.001*	Negative, good, statistically significant correlation between Quantitative NS1 (IU/dL) and TLC
Quantitative NS1 (IU/dL) and Platelets Count	-0.526	0.001*	Negative, fair, statistically significant correlation between Quantitative NS1 (IU/dL) and Platelets Count
Quantitative NS1 (IU/dL) and Pulse Pressure (mm Hg)	-0.382	0.001*	Negative, poor, statistically significant correlation between Quantitative NS1 (IU/dL) and Pulse Pressure (mm Hg)
Quantitative NS1 (IU/dL) and SGOT (IU/L)	-0.205	0.001*	Negative, very poor, statistically significant correlation between Quantitative NS1 (IU/dL) and SGOT (IU/L)
Quantitative NS1 (IU/dL) and SGPT (IU/L)	-0.135	0.024*	Positive, very poor, statistically significant correlation between Quantitative NS1 (IU/dL) and SGPT (IU/L)

TLC: Total Leucocyte count, SGPT: Serum glutamic pyruvic transaminase, SGOT: Serum glutamate oxaloacaetic transaminase

Table 3: Association Between Quantitative ELISA and Complications

Complications	Present	Absent	Total
Quantitative ELISA Result			
Negative	0	22	22
	0.0%	100%	100%
Positive	27	231	258
	10.5%	89.5%	100%
Total	27	253	280
	9.6%	90.4%	100.0%

Pearson Chi-square test applied.
Chi-square value=2.548, df=1, *P*-value=0.110, Not significant.
ELISA: Enzyme-linked immunosorbent assay.

Table 4: Association Between Quantitative ELISA and Mortality Rate

Mortality Rate	High	Low	None	Total
Quantitative ELISA Result				
Negative	0	8	14	22
	0.0%	36.4%	63.6%	100%
Positive	53	57	148	258
	20.5%	22.1%	57.4%	100%
Total	53	65	162	280
	18.9%	23.2%	57.9%	100%

Pearson Chi-square test applied.
Chi-square value=6.436, df=2, *P*-value=0.040, Significant.
ELISA: Enzyme-linked immunosorbent assay.

Table 5: Association Between Quantitative ELISA and Clinical Outcome

Clinical Outcome	Death	Discharge	Total
Quantitative ELISA Result			
Negative	0	22	22
	0.0%	100.0%	100%
Positive	5	253	258
	1.9%	98.1%	100%
Total	5	275	280
	1.8%	98.2%	100.0%

Pearson Chi-square test applied.
Chi-square value=0.434, df=1, *P*-value=0.510, Not significant.
ELISA: Enzyme-linked immunosorbent assay.

DISCUSSION

Dengue fever is one of the most serious infectious diseases, wreaking havoc on public health in many tropical and subtropical nations. The chance of acquiring a secondary dengue infection is directly proportional to increasing age. Aging also impairs physiological functions affecting the immune system and increasing the presence of chronic diseases.[7] In a study by Gitika *et al.* where a little over half of the patients (52%) were in age group of 21–40 years, 14% of patients were <10 years, 24% of patients belonged to 41–60-year age group and >60-year age group showed 10% of patients. The mean age was 37.31 ± 17.54 years. Most of these patients were adults because they form the working-age group and are more exposed to insect bites[8] which is in similarity with our study having most patients within the age group 21–40 years. Another similar study was done by Savargaonkar *et al.* in which the most afflicted age group was found to be between 11 and 30 years.[9]

In a study conducted by Muhammad *et al.*, where dengue fever incidence in 95 patients was observed, they found 81.1% males and 18.9% females.[10] This is in similarity with the gender differences in our study that is, male 68% and female 32%. In another similar study by Tahlan *et al.*, a male-to-female ratio of 2:1 was observed. This might be due to a reason that males predominantly form the working population and more prone to infection by mosquito bites in the daytime.[11]

Fever had been identified as the most prevalent presenting symptom by Neeraja *et al.* and Shah *et al.* in 100% of their study population. However, Ghosh *et al.* in his study found 71% of patients presenting with fever and chills.[12-14] Jelinek *et al.* state the symptoms commonly associated with dengue, such as fever (86%), myalgia (85.6%), headache (59.2%) arthralgia and exanthema can help make the diagnosis, when present, but the absence of typical symptoms does not exclude infection. Most patients with dengue in this study were symptomatic and reported fever.[15]

When compared to cases without comorbidities, comorbidities elevated the case fatality rates (CFRs) of hospitalised dengue by 3-–17-fold. Furthermore, independent of dengue severity or age, the CFRs for hospitalised dengue were greater in the presence of common comorbidities.[16] In a study by Lee *et al*., the impacts of coexisting comorbidity(ies) and glycaemic control on dengue severity were emphasised. As there were no comorbidities associated among the subjects who had tested positive and negative for the ELISA test, the findings mentioned above can be related to our study.[17]

Godbole *et al*., in their study, found pleural effusion and ascites in 11% of the dengue patients. About 11% of the individuals experienced bleeding, which is greater than the findings from our study (4.3%).[18] Numerous studies have pointed out common and uncommon complications associated with dengue fever. These include acute respiratory distress syndrome, encephalopathy and encephalitis, swollen lymph nodes, enlarged spleen, myocarditis, anaemia, multiple organ failure, hepatitis, fever, refractory shock, portal hypertension, pericardial effusion, muscle inflammation (myositis), acute kidney injury and disseminated intravascular coagulopathy.[19-21]

Eight patients (11.1%) died in a study by Chandralekha *et al*. of 72 critically ill dengue patients.[22] In their investigation, Juneja *et al*. reported a mortality rate of 6.1%.[23] In a multicentre study by Schmitz *et al*., 42 individuals in India who were brought to the intensive care unit (ICU) with dengue haemorrhagic fever or dengue shock syndrome reported 8 in-ICU deaths, resulting in a 19% mortality rate.[24] In-ICU and in-hospital death rates were reported to be 18.6% and 19.6%, respectively, in a study by Amâncio *et al*.[25] The variations in disease severity could be the cause of the differences between our study results and other findings.

Many studies have been conducted to investigate the utility of liver transaminase levels, platelet counts and other clinical and laboratory parameters in predicting severe dengue, and it has been demonstrated that none of these parameters can be used alone to predict severe dengue.[26-29] According to Paranavitane *et al*., NS1 antigen levels are significantly and inversely related to all white blood cell parameters. Serum NS1 antigen levels were found to be significantly ($P = 0.0001$) and inversely related to total white cell and lymphocyte counts.[30] However, according to Ju and Brasier, platelet and lymphocyte counts, as well as serum interleukin-10 levels, were the most important variables associated with severe dengue.[31] Although Duyen *et al*. did not correlate the kinetics of NS1 antigen levels with overall clinical disease severity, they did show that higher NS1 antigen levels on day 3 of infection were associated with lower platelet counts.[32] Similarly, a negative and statistically significant correlation was observed between quantitative NS1 with TLC, platelet count, pulse pressure and SGOT.

CONCLUSION

This study underscores the significance of early and precise diagnosis in managing dengue fever. Our findings highlight the utility of quantitative estimation of dengue NS-1 antigen by ELISA, demonstrating strong correlations with clinical and laboratory parameters. The high positivity rates observed with quantitative ELISA NS1 tests, alongside the notable mortality rates, emphasise the severity of dengue infections. Importantly, the significant correlations observed between NS1 antigen levels and various clinical indicators, such as total leucocyte count, platelet count and liver enzymes, provide valuable insights into disease progression and prognosis. These results underscore the importance of comprehensive patient evaluation and monitoring in mitigating the adverse outcomes associated with dengue fever. Further research and clinical validation are warranted to enhance our understanding of dengue pathogenesis and refine diagnostic and management strategies to improve patient outcomes.

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Ethical approval

The research/study was approved by the Institutional Review Board at Lokmanya Tilak Municipal Medical College and General Hospital, Sion, Mumbai, number IEC/DISS/17118, dated 7th December, 2018.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent.

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Conflicts of interest

There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

REFERENCES

1. Harapan H, Michie A, Sasmono RT, Imrie A. Dengue: A Minireview. Viruses 2020;12:829.
2. Chatchen S, Sabchareon A, Sirivichayakul C. Serodiagnosis of Asymptomatic Dengue Infection. Asian Pac J Trop Med 2017;10:11-4.
3. Peeling RW, Artsob H, Pelegrino JL, Buchy P, Cardosa MJ, Devi S, *et al*. Evaluation of Diagnostic Tests: Dengue. Nat Rev Microbiol 2010;8:S30-7.
4. Stanaway JD, Shepard DS, Undurraga EA, Halasa YA, Coffeng LE, Brady OJ, *et al*. The Global Burden of Dengue: An Analysis from the Global Burden of Disease Study 2013. Lancet Infect Dis 2016;16:712-23.
5. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, *et al*. The Global Distribution and Burden of Dengue. Nature 2013;496:504-7.
6. Haroon M, Jan H, Faisal S, Ali N, Kamran M, Ullah F. Dengue Outbreak in Peshawar: Clinical Features and Laboratory Markers of Dengue Virus Infection. J Infect Public Health 2019;12:258-62.
7. Thai KT, Nishiura H, Hoang PL, Tran NT, Phan GT, Le HQ, *et al*. Age-specificity of Clinical Dengue During Primary and Secondary Infections. PLoS Negl Trop Dis 2011;5:e1180.
8. Gitika G, Garg M, Gill GS. Evaluation of Hematological and Biochemical Profile of Early Dengue Patients. Int J Contemp Med Res 2018;5:F1-4.
9. Savargaonkar D, Sinha S, Srivastava B, Nagpal BN, Sinha A, Shamim A, *et al*. An Epidemiological Study of Dengue and its Coinfections in Delhi. Int J Infect Dis 2018;74:41-6.
10. Aamir M, Masood G, Aamir W, Rasheed A, Ejaz A, Syed S. Gender Difference in Patients with Dengue Fever Admitted in a Teaching Hospital, Lahore. PJMHS 2014;8:12-5.
11. Tahlan A, Bhattacharya A, Singla N, Singh R. Haematological Profile of Dengue Fever. Med Sci Monit 2017;5:5367-71.
12. Neeraja M, Lakshmi V, Teja VD, Umabala P, Subbalakshmi MV. Serodiagnosis of Dengue Virus Infection in Patients Presenting to a Tertiary Care Hospital. Indian J Med Microbiol 2006;24:280-2.
13. Shah GS, Islam S, Das BK. Clinical and Laboratory Profile of Dengue Infection in Children. Kathmandu Univ Med J 2006;4:40-3.
14. Ghosh G, Urhekar AD, Kosta S. A Clinicomicrobiological Study of Dengue Fever Cases in a Tertiary Care Centre of Navi Mumbai. Int J Bioassays 2013;2:1462-7.
15. Jelinek T, Mühlberger N, Harms G, Corachán M, Grobusch MP, Knobloch J, *et al*. Epidemiology and Clinical Features of Imported Dengue Fever in Europe: Sentinel Surveillance Data from TropNetEurop. Clin Infect Dis 2002;35:1047-52.
16. Macias AE, Werneck GL, Castro R, Mascareñas C, Coudeville L, Morley D, *et al*. Mortality among Hospitalized Dengue Patients with Comorbidities in Mexico, Brazil, and Colombia. Am J Trop Med Hyg 2021;105:102-9.
17. Lee IK, Hsieh CJ, Lee CT, Liu JW. Diabetic Patients Suffering Dengue are at Risk for Development of Dengue Shock Syndrome/Severe Dengue: Emphasizing the Impacts of Co-Existing Comorbidity(ies) and Glycemic Control on Dengue Severity. J Microbiol Immunol Infect 2020;53:69-78.
18. Godbole V, Rana H, Mehta K, Gosai F. Rising Trend of Cases of Dengue Fever Admitted in a Tertiary Care Hospital in Vadodara – A Retrospective Study. Apollo Med 2014;11:255-60.
19. Fujimoto DE, Koifman S. Clinical and Laboratory Characteristics of Patients with Dengue Hemorrhagic Fever Manifestations and their Transfusion Profile. Rev Bras Hematol Hemoter 2014;36:115-20.
20. Pothapregada S, Kamalakannan B, Thulasingam M. Clinical Profile of Atypical Manifestations of Dengue Fever. Indian J Pediatr 2016;83:493-9.
21. Carod-Artal FJ, Wichmann O, Farrar J, Gascón J. Neurological Complications of Dengue Virus Infection. Lancet Neurol 2013;12:906-19.
22. Chandralekha, Gupta P, Trikha A. The North Indian Dengue Outbreak 2006: A Retrospective Analysis of Intensive Care Unit Admissions in a Tertiary Care Hospital. Trans R Soc Trop Med Hyg 2008;102:143-7.
23. Juneja D, Nasa P, Singh O, Javeri Y, Uniyal B, Dang R. Clinical Profile, Intensive Care Unit Course, and Outcome of Patients Admitted in Intensive Care Unit with Dengue. J Crit Care 2011;26:449-52.
24. Schmitz L, Prayag S, Varghese S, Jog S, Bhargav-Patil P, Yadav A, *et al*. Nonhematological Organ Dysfunction and Positive Fluid Balance are Important Determinants of Outcome in Adults with Severe Dengue Infection: A Multicenter Study from India. J Crit Care 2011;26:441-8.
25. Amâncio FF, Heringer TP, de Oliveira CC, Fassy LB, de Carvalho FB, Oliveira DP, *et al*. Clinical Profiles and Factors Associated with Death in Adults with Dengue Admitted to Intensive Care Units, Minas Gerais, Brazil. PLoS One 2015;10:e0129046.
26. Lee LK, Gan VC, Lee VJ, Tan AS, Leo YS, Lye DC. Clinical Relevance and Discriminatory Value of Elevated Liver Aminotransferase Levels for Dengue Severity. PLoS Negl Trop Dis 2012;6:e1676.
27. Diaz-Quijano FA, Villar-Centeno LA, Martinez-Vega RA. Predictors of Spontaneous Bleeding in Patients with Acute Febrile Syndrome from a Dengue Endemic Area. J Clin Virol 2010;49:11-5.
28. Thomas L, Brouste Y, Najioullah F, Hochedez P, Hatchuel Y, Moravie V, *et al*. Predictors of Severe Manifestations in a Cohort of Adult Dengue Patients. J Clin Virol 2010;48:96-9.
29. Srikiatkhachorn A, Green S. Markers of Dengue Disease Severity. Curr Top Microbiol Immunol 2010;338:67-82.
30. Paranavitane SA, Gomes L, Kamaladasa A, Adikari TN, Wickramasinghe N, Jeewandara C, *et al*. Dengue NS1 Antigen as a Marker of Severe Clinical Disease. BMC Infect Dis 2014;14:570.
31. Ju H, Brasier AR. Variable Selection Methods for Developing a Biomarker Panel for Prediction of Dengue Hemorrhagic Fever. BMC Res Notes 2013;6:365.
32. Duyen HT, Ngoc TV, Do TH, Hang VT, Kieu NT, Young PR, *et al*. Kinetics of Plasma Viremia and Soluble Nonstructural Protein 1 Concentrations in Dengue: Differential Effects According to Serotype and Immune Status. J Infect Dis 2011;203:1292.

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