

Original research article**Early detection of dengue by ELISA****¹Lahiri Somsubhra, ²Narmadha E, ³Reddy Spoorti Channa**^{1,2}Assistant Professor, Department of Microbiology, Anna Medical College, Mauritius³Junior Resident, Department of Medicine, Dr. S.S. Tantia Medical College, Hospital and Research Centre, Sri Ganganagar, Rajasthan, India**Corresponding Author:**

Narmadha E

Abstract

It is essential to have an accurate and early diagnosis of dengue in order to diagnose dengue virus infections in a timely manner. Through the utilisation of NS1 antigen and IgM antibody as a fast diagnostic tool, the purpose of this work is to identify dengue in its earliest stages. In all, 108 serum samples were collected for the IgM Ab detection of dengue virus infection, whereas 101 serum samples were collected for the NS1 Ag detection of dengue virus infection. Using independent ELISA kits, these samples were analysed to determine whether or not they contained NS1 antigen and IgM antibodies. 19 out of 101 samples were positive for the presence of NS1. There were 33 samples that tested positive for IgM out of a total of 108. There were 36 positive results across both the 101 and 108 samples. According to the findings, the ELISA method that was used is suitable for the early detection of dengue and has the potential to boost the diagnostic effectiveness for early diagnosis.

Keywords: Dengue virus, IgM, ELISA, antibodies, infection

Introduction

Infection with the dengue virus is a disease that is spread by mosquitoes and is caused by a virus with a single strand of positive sense RNA that is a member of the flaviviridae family. It is a sickness that is becoming more common in tropical as well as subtropical regions. More than 90 nations in Southern Asia and America, Eastern Mediterranean, Western Pacific, and Africa are affected by the disease, which poses a threat to billions of people worldwide ^[1]. As a result of the rapid emergence of disease and the accompanying increase in the incidence and spread of disease, thousands of people die around the world every year. The virus encodes five unique antigenically related serotypes that induce a range of clinical sickness ranging from fever to deadly illness, dengue shock syndrome, and hemorrhagic fever. These serotypes are responsible for the virus's ability to cause these illnesses. The severity of the condition is used to categorise patients, and Fever, discomfort in the muscles and joints, pain in the brain, nausea, vomiting, and hemorrhagic symptoms are all clinical hallmarks of the disease. According to the findings of numerous population-based studies, the most common consequence of dengue virus infection is the development of asymptomatic illnesses ^[2,3]. Because the disease can advance rapidly even in the absence of symptoms, a laboratory diagnosis that is done with tests that are quick, accurate, and cost-effective is essential ^[4]. Because diagnostic laboratories in many underdeveloped nations do not have the facilities to diagnose dengue, it is imperative to identify dengue in its early stages by employing NS1 antigen and IGM antibody. The quick diagnosis is necessary in order to reduce the risk of complications caused by the dengue virus, particularly in areas where dengue is more likely to occur. The *Aedes aegypti* mosquito is characterised by its black colouring, tiny size, banded legs, and lyre-shaped patterns on its body. It is common in tropical and subtropical places all over the world and has traditionally been regarded as a key vector for viral infections such as dengue fever, chikungunya fever, and yellow fever. It often feeds during the day and lays its eggs on containers used for storage; these eggs have the potential to survive for longer periods of time and spread to other locations ^[5]. With the use of enzyme-linked immunosorbent test, NS 1 ANTIGEN, a non-structural protein that was initially discovered in 2006, enables quick detection on the very first day of fever, even before the development of antibodies. There are five different serotypes of the dengue virus: DEN 1, 2, 3, 4, 5. Of these, the three most common are the NS proteins (NS 1, NS 3, and NS 5) that are detected in flavivirus-infected cells. NS 1 is a glycoprotein that plays a critical role in the formation of the morphology of viral replication. This glycoprotein is required for the survivability of the virus. During the acute phase of the infection, it has been detected in the bloodstream of the majority of individuals who have been examined. The category of antibodies known as 6 IgM antibodies is the largest one that can be produced by vertebrates. It is the response that occurs immediately after the first encounter with an antigen. The presence of these IgM antibodies is diagnostic of a primary infection that has occurred during the past few weeks, and the detection of these antibodies will assist in the early diagnosis of dengue. Therefore, the purpose and goal

of the current investigation is to predominantly identify NS1 antigen and IgM antibody in patients for the purpose of making an early diagnosis of dengue. Second, to provide the most sensitive diagnostic method for dengue, which makes it possible to begin supportive medication and monitor the risk of complications such as dengue hemorrhagic fever earlier.

Materials and Methods

The Department of Medicine in association with microbiology at the Dr. S. S. Tania Medical College, Hospital, and Research Centre in India was responsible for carrying out this research. Patients who had clinical suspicions of having dengue were recruited to provide their serum for testing for the presence of NS1 and IgM Ab respectively, and these samples were collected and analysed during the course of the study. By using ELISA, each sample was analysed to determine whether or not it contained anti-dengue IGM antibodies and NS1 antigen. The National Institute of Virology in Pune was the source for the kits that were purchased. In order to carry out this investigation, a total of 17 serum samples and 17 serum panels were collected.

Results

Table 1: Sensitivity and reliability of the dual method

Dengue diagnostics	Sensitivity %, [95% CI]	P value (between group)
	(N = 17)	
SD Dengue Duo NS1	46.44–94.71	0.14
SD Dengue Duo IgM	15.10–67.26	0.44
SD Dengue Duo NS1/IgM	62.15–100	0.17

Table 2

Dengue diagnostics	Sensitivity, % (N = 17) (95% CI) [±]	Specificity, % (N = 17) (95% CI) [±]	Efficiency, % (N = 17) (95% CI)	Positive predictive value, % (95% CI)	Negative predictive value, % (95% CI)
SD Dengue Duo NS1/IgM	84.0–93.16	96.29–100	88.21–95.28	91.82–100	71.92–86.71

Discussion

The dengue virus is a member of the family Flaviviridae and the genus Flavi virus. It is an RNA virus that contains a single strand. There are other major vector-borne viruses, such as the yellow fever virus and the St. Louis encephalitis virus, which are all members of the same family. The bite of an Aedes mosquito is the primary route through which the dengue virus is transmitted from mosquitoes to humans. The disease can be passed on by contact with infected blood or through exposure in healthcare settings, such as through the use of needles or through instances of vertical transmission [7]. It has been observed that the global prevalence of dengue fever has increased by a factor of thirty over the past fifty years. This rise can be attributed, in large part, to migration in population, increasing travel and inadequate vector control measures [8]. In India, the diagnosis of dengue fever is most frequently carried out with the assistance of quick kit-based serological testing. The usage of ELISA facilities was difficult to get at the majority of the diagnostic institutes, and the availability of alternative diagnostic kits on the market was unregulated by both national and international testing bodies. Because of this, there is a worrying lack of independent testing of the diagnostic accuracy promised by manufacturers, which may result in incorrect diagnoses of dengue infection. The bulk of the cases that were evaluated for IgM detection in the study revealed that the study's range, specificity, and overall accuracy were significantly greater and more consistent. For the purpose of laboratory diagnosis of dengue virus infection, dengue viremia and antibody response patterns are the most commonly available tools. The peak of dengue viremia emerges early in the febrile phase, which has been demonstrated to correspond with the severity of the disease. The degree of viremia has consequences for case treatment as a result of this correlation. The detection of NS 1 Ag and IgM antibodies has been the primary method for diagnosing dengue infection [9] for quite some time. It has been demonstrated that NS 1 is a very specific viral marker, which makes it exceptionally trustworthy for the diagnosis of dengue infection from the very first day of fever onwards. NS 1 emerges prior to seroconversion and can be detected from day 1 to day 6. The detection of this NS 1 Ag and IgM antibodies has become the key diagnostic criterion for a dengue infection, and it appears prior to seroconversion [10].

Conclusion

It is interesting to note that the current findings imply that ELISA, which is utilised for the detection of NS1 antigen, has become a viable and specific method for the diagnosis of acute dengue infection. Therefore, using NS1 antigen in conjunction with the dengue IgM test could greatly improve the accuracy of the diagnosis of infection.

References

1. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, Drake JM, Brownstein JS, Hoen AG, Sankoh O, Myers MF, George DB, Jaenisch T, Wint GR, Simmons CP, Scott TW, Farrar JJ, Hay SI. The global distribution and burden of dengue. *Nature*. 2013 Apr 25; 496(7446):504-7.
2. Normile D Tropical medicine. Surprising new dengue virus throws a spanner in disease control efforts. *Science*. 2013 Oct 25; 342(6157):41
3. Blacksell SD, Newton PN, Bell D, Kelley J, Mammen MP Jr, Vaughn DW, Wuthiekanun V, Sungkakum A, Nisalak A, Day NP The comparative accuracy of 8 commercial rapid immune chromatographic assays for the diagnosis of acute dengue virus infection *Clin Infect Dis*. 2006 Apr 15; 42(8):1127-34
4. Blacksell SD, Jarman RG, Bailey MS, Tanganuchitcharnchai A, Jenjaroen K, Gibbons RV, *et al*. Evaluation of six commercial point-of-care tests for diagnosis of acute dengue infections: the need for combining NS1 antigen and IgM/IgG antibody detection to achieve acceptable levels of accuracy. *Clin Vaccine Immunol* 2011;18:2095-101.
5. Brady OJ, Gething PW, Bhatt S, Messina JP, Brownstein JS, Hoen AG *et al*. Refining the global spatial limits of dengue virus transmission by evidence-based consensus. *PLoS Negl Trop Dis*. 2012; 6:e1760. doi:10.1371/journal.pntd.0001760.
6. Dwivedi, V. D., Tripathi, I. P., Tripathi, R. C., Bharadwaj, S., and Mishra, S. K. (2017). Genomics, proteomics and evolution of Dengue virus. *Briefings in functional genomics*. 16(4): 217–227.
7. Dengue. *Halstead SBLancet*. 2007 Nov 10; 370(9599):1644-52
8. Gupta E, Dar L, Narang P, Srivastava VK, Broor S. Serodiagnosis of dengue during an outbreak at a tertiary care hospital in Delhi. *Indian J Med Res*. 2005;121:36–8.
9. A Shrivastava, PK Dash, NK Tripathi :Evaluation of Dengue NS 1 ELISA Assay for early diagnosis of dengue infection. *Indian Journal of Medical Microbiology* vol 29; no 4; Jan 2011.
10. Chakravarti A, Kumar A, Malik S, “Detection of dengue infection by combining the use of NS1 antigen based assay with antibody detection”, *The Southeast Asian journal of tropical medicine and public health, Maulana Azad Medical College, New Delhi, India; 2011 March; 42(2):297-302.*
11. Datta S, Watal C, “Dengue NS1 antigen detection: a useful tool in early diagnosis of dengue virus infection”, *Indian journal of Medical Microbiology, Sir Ganga Ram Hospital, New Delhi, India; 2010 April-June; 28(2):107-10.*
12. Kassim FM, Izati MN, TgRogayah TA, Apandi YM, Saat Z, “Use of dengue NS1 antigen for early diagnosis of dengue virus infection”, *The Southeast Asian journal of tropical medicine and public health, Kuala Lumpur, Malaysia, 2011 May; 42(3):562-9.*