Dengue NS1 Antigen: A Tool in Early Detection of Dengue Virus Infection

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Abstract:

Objectives: This aim of the study was to evaluate the efficacy of NS1 antigen assay for early diagnosis of dengue virus infection in a tertiary care hospital.

Methods: This cross sectional study was carried out in medicine unit of Square hospital from May to August 2013. Total 100 patients with dengue fever were included. Complete blood count, alanine aminotransferase (ALT), aspartate aminotransferase (AST), Dengue NS1 antigen and IgM and IgG antibodies of dengue virus were done in all cases.

Results: Of the 100 sera tested, 75% were positive for dengue virus infection based on dengue NS1 antigen, IgM antibody and IgG antibody. Dengue NS1 antigen and IgM, IgG antibody were able to detect dengue virus infection between day 1 to day 8 in 92% of samples, 86.7% of samples and 82.6% of samples respectively. Sixty nine percent (69%) were found positive for dengue NS1 antigen, 65% were IgM positive and 62% were IgG positive. Based on the dengue NS1 antigen and IgM antibody combination, 74% were positive for dengue virus infections. Sensitivity of Dengue NS1 antigen was 92.3% and specificity of 74.28% in comparison to IgM antibody. Based on the antigen and IgG antibody combination, detection rate increased to 75%. Sensitivity of dengue NS1 antigen was 90.3% and specificity of 65.8% in comparison to IgG antibody.

Conclusion: Dengue NS1 antigen is a useful, sensitive and specific test for early diagnosis of dengue virus infection and it improves diagnostic efficiency in combination with antibody test.

Key words: Dengue fever, NS1 antigen.

Introduction:

Dengue fever (DF) is the most common arboviral illness in humans. Each year, an estimated 50-100 million cases of dengue fever and 500,000 cases of dengue hemorrhagic fever occur worldwide, with 30000 deaths (mainly in children). Globally 2.5-3 billion people in approximately 112 tropical and subtropical countries are at risk of dengue infection.[1,2] In Bangladesh an epidemic of DF broke out in June 2000 when it took heavy toll (5,555 cases and 93 deaths).[3,4] Due to its impact on high mortality, it is necessary to have a sensitive laboratory assay for early diagnosis of dengue

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virus infection. The diagnostic methods available are virus isolation, genomic RNA detection by reverse transcriptase PCR and serological test such as immunoglobulin M and G by enzyme linked immunosorbent assay (ELISA). The first two assays need specialized laboratory as well as well trained laboratory personnel which is not widely available. So dengue specific antibody test is the most commonly available method.[5] IgM antibodies are detectable approximately 4-6days after onset of fever. By day 5 , it became detectable in 80% cases and 93-99% cases by day 6 to 10 which delay in early diagnosis of dengue infection.[6] Dengue virus non-structural protein 1(NS1) antigen, a highly structured glycoprotein, produced both in membrane associated and secretory forms is detectable in blood from first day after onset of fever up to day 9.[7] So detection of dengue NS1 antigen represents a new approach to the diagnosis of acute dengue virus (DV) infection. In our study we aimed to evaluate the NS1 antigen assay for early diagnosis of dengue virus infection in a tertiary care hospital.

Materials and Methods:

This cross sectional study was carried out in medicine unit of Square Hospital from May to August 2013. Total 100 patients with DF were included. DF was defined according

to National guideline of Bangladesh. The demographic profile was taken from each case. Complete blood count, alanine aminotransferase (ALT), aspartate aminotransferase (AST), Dengue NS1 antigen and IgM and IgG antibodies of dengue virus were done in all cases. Other relevant investigations were done to determine complications or to exclude differential diagnosis when required.

Results:

In this study, total 100 patients with DF were included with male, female ratio 1:0.8. Majority of the cases (86%) were below 50 years of age. About 30% cases were students and 22% were service holders. More than 50% cases came from Dhanmondi area. Total 85% patients were classical dengue fever and 15% of dengue hemorrhagic fever. Only 2% complications were noted and there were no adverse outcome.

Leucopenia was noted in 59% cases and mean WBC was 3677.88±1382.05 SD. Total platelet count was found < 100,000/cmm in all patients and it was <10,000/cmm in 12% patients. Mean ALT of the study population was 168.43± 213.12 SD and mean AST was 213.86+322.62. Dengue NS1 antigen was positive in 69% cases. IgM antibody to dengue virus was positive in 65% cases and IgG in 62% cases.

Of the 75 dengue positive samples, dengue NS1 antigen and IgM, IgG antibody were able to detect dengue virus infection between day 1 to day 8 in 92% of samples (69/75), 86.7% of samples (65/75) and 82.6% (62/75) of samples respectively.

Combination of Dengue NS1 antigen and antibody tests:

The results of Dengue NS1 antigen were compared to the results of dengue antibody tests (IgM and IgG). Total 69 samples were positive for IgM, giving the serological test a detection rate of 69%. Based on the combination of dengue NS1 antigen and IgM antibody test, total 74 patients (74%) were positive for dengue virus infections. Dengue NS1 antigen was not detected in 7.69% (5/65) of IgM positive samples but it was detected in 25.7% (9/35) of IgM negative samples. So sensitivity of Dengue NS1 antigen was 92.3% and specificity of 74.28% in comparison to IgM antibody.

Table I

Comparison of Dengue NS1 antigen and dengue antibody IgM

		Dengue antibody (IgM)	
		Positive (n=65)	Negative (n=35)
Dengue NS1	Positive (n=69)	60	9
antigen	Negative (n=31)	5	26

Dengue NS1 antigen was also compared to dengue antibody IgG. A total of 62% samples were positive giving detection rate of 62%. Based on the antigen and IgG antibody combination, detection rate increased to 75%. Dengue NS1 antigen was not detected in 9.6% (6/62) of IgG positive samples but detected in 34.2% (13/38) of negative samples. So sensitivity of dengue NS1 antigen was 90.3% and specificity of 65.8% in comparison to IgG antibody.

Table-II

Comparison of Dengue NS1 antigen and dengue antibody IgG

		Dengue antibody (IgG)	
		Positive (n=62)	Negative (n=38)
Dengue NS1	Positive (n=69)	56	13
antigen	Negative (n=31)	6	25

Discussion:

With the escalating incidence of dengue and the absence of vaccine for prevention of the disease, it is important to detect early dengue virus (DENV) infection for management of patients as well as for effective public health control of dengue outbreaks. Various studies have confirmed the detection of dengue NS1 antigen is useful for early diagnosis of dengue infections.[8,9] Of the 100 patients tested, 69% patients were positive on dengue NS1 antigen, 65% were IgM positive and 62% were IgG positive. Serological testing has always been the method of choice in most laboratory settings. In this study, the detection of DENV antibodies, IgM and /or IgG antibody tests, were found in 67% cases. Combination of antigen test with antibody were analysed to determine the performance in detecting DENV infections. Based on the combination of dengue NS1 antigen and IgM, total 74 patients (74%) were positive for dengue virus infections. Dengue NS1 antigen was not detected in 7.69% (5/65) of IgM positive samples but it was detected in 25.7% (9/35) of IgM negative samples. So sensitivity of Dengue NS1 antigen was 92.3% and specificity of 74.28% in comparison to IgM antibody. Based on the antigen and IgG antibody combination, detection rate increased to 75%. Dengue NS1 antigen was not detected in 9.6% (6/62) of IgG positive samples but detected in 34.2% (13/38) of negative samples. So sensitivity of dengue NS1 antigen was 90.3% and specificity of 65.8% in comparison to IgG antibody. So the combination of antigen and antibody test gave a better yield for detecting dengue infections. A study conducted by Kassim et.al in 2011 was analyzed which revealed that

sensitivity of dengue NS1 antigen was 42.05% and specificity of 78.21% when compared to dengue antibody. [10]

Dengue NS1 antigen has allowed for early detection of DENV infection as the antigens remain detectable in blood for 5 days after onset of fever and rapidly disappear after formation of specific antibodies.[11] In this study, DENV antigens were detected as early as day1 to day 8 of fever. It is comparable to a study by Alcon et al published in 2006 who recovered NS1 antigen up to day 9 of symptoms.[12] A study conducted by Koraka et al in 2003 showed that antigen detection was low in endemic area due to formation of immune complexes following a secondary dengue infection and inappropriate temperature storage of samples.[13] So it is important that samples are collected, stored and transported properly to maintain the quality and stability of samples tested.

Dengue NS1 antigen assay is simple and easily to perform especially in laboratories that are already performing ELISA. Within a few hours, the physician can detect DENV infection in outpatient clinic and early detection will enable was not only in patient management but also notify public health authorities. However, it was unable to distinguish between serotype of dengue virus which was possible by PCR. As most laboratories have limited funds to set up PCR tests, NS1 antigen should be considered as an additional diagnostic tool for early dengue virus infection. The combination of antigen and antibody tests may increase the diagnostic efficiency on DENV infection. This is in comparable with Shekaran et al in 2007 who showed combination of tests were sensitive and specific in endemic settings.[14]

Conclusion:

The evaluation of Dengue NS1 antigen assay shows that it is useful, sensitive and specific test for early diagnosis of dengue virus infection in the laboratories that have limited resources, lack viral culture or RT-PCR facilities.

Conflict of Interest: None

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