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COMPARISION OF DIAGNOSTIC EFFICACY OF NS1 ANTIGEN BASED IMMUNOCHROMATOGRAPHIC TEST WITH IMMUNO-SORBENT ASSAY AND ITS ROLE IN DETECTION OF EARLY DENGUE INFECTION

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ABSTRACT:INTRODUCTION: Dengue is an acute viral infection with potential fatal complications. Rapid and easy diagnosis of dengue can help in patient triage and care-management. The detection of dengue virus NS1 antigen by rapid lateral flow tests offers a faster method to a presumptive diagnosis in the peripheral centers of developing countries like India where the laboratory has no great technological backup. **MATERIALS AND METHODS:** A total of 351 sera from patients suspected of having dengue virus infection were tested for dengue NS1 antigen and dengue IgM and IgG antibodies by both ELISA and immunochromatographic test (ICT). **RESULTS:** From the 351 samples tested, 249 (70.94%) of the sera were found to be positive for DENV infection based on the IgM antibody or IgG antibody or NS1 antigen. Of the 249 samples 105 (42.16%) were positive for only IgM or only IgG or both antibodies by ELISA and 101 (40.56%) by ICT. Based on dengue NS1 antigen along with IgM and/or IgG tests, 144 (57.8%) were positive for dengue infections by ELISA and 139 (55.8%) by ICT. Among these 144 samples, only dengue NS1 antigen was detected in 67 (26.90%) by ELISA and 65 (26.10%) by ICT. **CONCLUSIONS:** Inclusion of NS1 in the diagnosis of dengue increases the detection rate significantly. The sensitivity of ICT for both antigen and antibody detection are almost equal to ELISA. Thus, the potential use of the NS1 antigen along with antibody tests in an ICT could increase the diagnostic efficiency for early diagnosis of dengue infection.
KEY WORDS: Dengue, NS1 antigen, ICT, Immunosorbent assay.

INTRODUCTION: Dengue is a mosquito borne arboviral disease caused by genus Flavivirus and transmitted to humans through the bites of infected Aedes mosquitoes, principally Aedes. aegypti.[1] It is a major public health problem in tropical and subtropical countries.[2] Over 2.5billion people over 40% of the world's population are now at risk from dengue. Annually, the World Health Organization estimates that 50–100 million people are infected with dengue virus worldwide with estimated 500,000 cases of dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS) with about 12,500 deaths occurring.[3] Many dengue fever (DF) cases are self-limiting but its complications like haemorrhage and shock can be life-threatening. If untreated, mortality from the complications of DF is as high as 20%, whereas if recognized early

and managed properly, mortality is less than 1%.[4] There are three main laboratory methods to diagnose dengue infection: viral isolation in culture, detection of viral RNA, and specific IgM/IgG antibodies in paired sera. The gold standard is usually a combination of these methods.[5,6] However, methods such as virus isolation and genomic RNA detection (PCR), need a specialized laboratory, well trained laboratory personnel and costly, which are not widely available in hospital settings. On the contrary, there are commercially available immunochromatographic and ELISA tests for the detection of IgM/IgG antibodies which give results within few minutes to hours. But, the detection of antibodies in a dengue infected person is only possible after 4-5 days of disease onset.[7] Dengue NS1 antigen, a highly conserved glycoprotein, produced in both membrane-associated and secretion forms, is abundant in the serum of patients during the early stages of dengue infection.[8] Several studies conducted revealed the importance of dengue NS1 antigen as a biomarker; these antigens can be detected before the formation of antibodies.[9,10] NS1 antigen is detectable in blood from the first day after the onset of fever up to Day 9; and in the presence of IgM antibodies.[11] The main objective of this research was to compare the immuno-chromatographic test and ELISA for NS1 antigen, so as to improve the early detection of dengue in rural centers.

MATERIALS AND METHODS: In this study a total of 351 sera were collected from patients with suspected dengue infection. These patients were selected based on a clinical diagnosis of dengue infection and fulfilled the WHO case definition for dengue fever. The status of dengue infection in these patients was determined by serological detection of dengue IgM and IgG and detection of dengue NS1 antigen. All samples were tested using a commercially available immuno-chromatographic test and ELISA (Panbio for NS1, J. Mitra India for IgM and IgG antibodies) for NS1 antigen, IgM and IgG antibodies (J. Mitra, India). The test procedures were performed according to manufacturers' instructions.

RESULTS: Out of 351 samples tested, 70.94% (249/ 351) of the sera were found to be positive for Dengue virus infection based on the IgM antibody or IgG antibody or NS1 antigen or a combination of them.

COMBINATION OF DENGUE NS1 ANTIGEN AND ANTIBODY TESTS: The results of dengue NS1 antigen detection were compared to the results of the dengue antibody tests (IgM and/or IgG) and also compared both method rapid test and ELISA for antibodies and NS1 antigen. Of the 249 samples 42.16% (105/249) were positive for only IgM and/or IgG antibodies by ELISA and 40.56% (101/249) by ICT. Based on dengue NS1 antigen and/ or IgM and/or IgG tests, 57.8% (144/249) were positive for DENV infections by ELISA and 55.8% (139/249) by ICT. IgM and IgG dengue antibodies were found positive in 20.88% (52/249) of sample by ELISA. Only dengue NS1 antigen was detected in 26.90% (67/249) by ELISA and 26.10% (65/249) by ICT. See Figure 1.

COMPARISON OF DENGUE ICT AND ELISA TESTS: There was no significant difference found between detection of dengue infection by ICT and ELISA tests (p >1) see Table 1.

DISCUSSION: The techniques of dengue serologic diagnosis which have been widely used are based on the detection of dengue-specific IgM/IgG antibodies by ELISA. The dengue-specific antibodies begin to appear only around fifth day of fever in primary infection.[12] Even in most

secondary infections, both the IgM and IgG type antibodies cannot be recorded before third day.[13]So, there is a window period, both in primary and secondary dengue infection when only antibodies are tested. This window period can be reduced by using a highly specific and extremely reliable viral marker i.e dengue NS1antigen, which is detectable in blood from the first day after the onset of fever, both in primary and secondary infections.[14] In our study, of the 249 cases, 26.90% (67/249) were positive for only NS1 antigen. Considering very high specificity of NS1 it can be stated that we would have missed the diagnosis in 26.90% cases had we not included NS1 in the test panel. Datta et al and Shrivastava et al have shown that NS1 was positive in 140 out of 600 (23.3%) and 15 out of 91 (16%) cases respectively in their studies.[15,16]

During the acute phase, the presence of IgM antibodies alone suggests primary infection and detection of newly formed IgM antibodies occurs after viremia ends or after fever subsides.[14] IgM antibodies are detectable approximately 4 to 6 days after the onset of fever. By Day 5 of symptoms, 80% of cases have detectable IgM antibodies and 93-99% of cases have detectable IgM by Days 6 to 10.[14] In this study 91.8% (147/249) of cases have detectable IgM by Days 6 to 10. Once IgM is detected, the level increases rapidly and appears to peak about 2 weeks after the onset of symptoms, then decreases to undetectable levels over 2 to 3 months. In primary infection IgG antibodies become detectable at 10–14 days and in secondary infection, IgG antibodies present at high levels before or simultaneously with the IgM antibody response. IgG antibodies increase to high levels within the first week of infection and decline slowly over 3 to 6 months. Among two antibodies, IgG is a less reliable marker in the diagnosis of dengue infection.[12] Both clinical and sub-clinical infections can produce IgG which may persist for several years affecting the interpretation of test results.[14]It is highly likely that IgG levels could be higher in endemic areas because of bites from infected mosquitoes. However, dengue-specific IgM is a very good indicator of recent infection. It may also be detectable in secondary dengue infection.[12] Utility of antibody in the diagnosis of infections relies mainly on rising titres, especially in the endemic areas. However, repeat testing of cases, for the same infection, when the first test is negative or sending samples for determination of rise in titre is 'almost never' utilized in clinical practice. When NS1 is positive, there is no need of repeat testing as it is a highly specific marker of dengue infection. NS1 alone or in combination with either IgM or IgG was positive in 144 cases (57.83% cases) in our study

The present study also compared simultaneously the performance of two commercially available ELISA and ICT based test for the early diagnosis of dengue showing in Table 1. There was no "statistically significant" (P >0.001) difference found between ELISA and ICT.
This study has been carried out at a rural tertiary care teaching hospital and because of limited facility viral culture and RT- PCR not done. Therefore, applying gold standard tests in studies related to viral infections is out of reach of these centres. Dengue is an infection that is present in urban, semi-urban and rural areas. Our healthcare system is extremely resource poor. Top class technological backup is available only at few elite laboratories situated in big cities. In the peripheral centres of developing countries like India where the laboratory has to function without great technological backup and still is expected to provide reasonable opinion to the clinician in the management of infections like dengue, ICT could be helpful in early diagnosis of dengue infection as this test is almost equally sensitive as ELISA.

CONCLUSION: Our findings suggest that the NS1 antigen is a specific tool for diagnosing every clinically suspected case of Dengue fever on day 1. The addition of a dengue NS1 component to

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the dengue detection test panel significantly improved diagnostic sensitivity above IgG and IgM antibody testing alone. Our study found no significant differences in sensitivities of ICT and ELISA tests. Immunochromatographic test being cost effective, and need less technical expertise especially in peripheral health care centres or laboratories that have limited resources is an excellent tool for detection of dengue fever.

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Table 1: Comparison of Dengue ICT and ELISA tests

Result	ICT	ELISA
Positive	240	249
Negative	111	102

Number of Samples

Figure 1: Comparision of various dengue specific parameters by ELISA and ICT tests