ORIGINAL ARTICLE

EVALUATION OF COMMERCIAL NEWER RAPID TEST FOR DETECTION OF EARLY ACUTE DENGUE INFECTION

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ABSTRACT

Introduction: We have done a study of evaluation of commercial newer rapid immunochromatographic test for diagnosis of early dengue infection. It detects both NS1 antigen and IgM antibody simultaneously.
Material and Methods: The test to detect dengue infection is done by rapid immunochromatographic test (ICT) which defect NS1 antigen and IgM antibody. [Advantage Dengue NS1 Ag and Ab Combi Card].
Results: Among 280 dengue suspected cases 78 were positive by rapid ICT. NS1 Among these 78 cases 57 were NS1 positive and 21 were IgM positive. All samples were tested for IgM ELISA.
Conclusion: Our findings support the use of diagnostic tools based on NS1 antigen detection positive for diagnosis of acute dengue virus infection. These rapid immunochromatographic tests allow rapid detection of the NS1 Ag and IgM antibody of dengue virus. Detection of the dengue NS1 Ag using the symptomatic phase of illness represents an important advanced in the diagnosis of dengue fewer. The immunochromatographic test Dengue NS1 antigen STRIP- The first rapid diagnostic test for early dengue virus infection- was highly sensitive and specific and would therefore be a suitable first line test in the field.

Keywords: Rapid test, Dengue, IGm, ELISA

INTRODUCTION

Dengue virus is a mosquito borne virus (family flaviviridae, genus: flavivirus). Four serotypes of Dengue virus cause disease in humans. Symptoms range from mild fever to fatal Dengue hemorrhagic fever. [1] Dengue virus is an enveloped positive sense RNA virus. The genomic RNA is approximately 11 kb in length and is composed of three structural protein genes that encode for nucleocapsid or core protein (C), a membrane – associated protein (M), an envelope protein (E) and seven nonstructural (NS) protein genes including NS1 protein. [2] Although early diagnosis is useful in triaging patients, it could have a central role in dengue case management at a future time when antiviral drugs for dengue – the subject of intense research interest – become available for clinical use. [3] Among the non-structural proteins, NS1 is a highly conserved glycoprotein which appears essential for virus replication, although no precise function has yet been assigned to it. During acute dengue virus infection, NS1 is found associated with intracellular organelles or is transported through the cellular secretory pathway to the cell surface. [4, 5, 6] The hexameric form of dengue virus NS1 protein was also found circulating in the sera of patients during the acute phase of the illness. [7] Early diagnosis plays in crucial role in fore casting an early warning of an epidemic and in undertaking effective vector control measures. The precise diagnosis is achieved either by isolating the virus or by identifying viral RNA through RT-PCR [5] or by serodiagnosis by detecting dengue specific IgM and IgG antibodies. [8] Both virus isolation and RT- PCR are time consuming and costly laboratory methods, Thus in a majority of cases the only feasible diagnosis is based on the detection of dengue antigen or antibodies. Detection of dengue NS1 antigen indicating early dengue infection. The NS1 antigen possesses not only group specific but also type specific determinants and has been recognized as an important Antigen in dengue infection. [6, 8] Antigen detection of nonstructural dengue antigens may be of

benefit for an early stage rapid diagnosis of infection due to its long half life in the blood. [9]

MATERIAL & METHODS

Dengue suspects were defined as patients presenting with acute febrile illness, rashes, bleeding tendencies, leucopenia or thrombocytopenia were evaluated according to WHO criteria for probable dengue infection. [10] Blood samples were collected from patients clinically suspected of dengue during Sept – 2010 to Oct – 2011 form GMERS, sola civil hospital, Ahmedabad. Serum separated from the samples & detection of dengue infection is done by rapid immunochromatographic test which detect NS1 antigen and IgM antibody. [Advantage Dengue NS1 Ag and Ab Combi Card]. All the samples were also evaluated by dengue IgM antibody capture ELISA. The serum samples were obtained from suspected dengue infection where initially tested with immunochromatography card tests a rapid test device, taking 20 minutes. If detect NS1 Ag and IgM antibody simultaneously. The test kit consist two devices, one device for detection of dengue NS1 antigen and second device for the differential detection of dengue IgM antibodies in human sera. Dengue NS1 antigen device contains two lines; "C" (control line) and "T" (dengue NS1 antigen test line). Test line is coated with anti-dengue NS1 Ag. When a sample is added to the devices Dengue NS1 antigen if present in the sample will bind to the anti-dengue NS1 gold colloid conjugate making antigen- antibodies complex. This complex migrates along the membrane to the test region and forms the visible pink line at "T" as antibody – antigen – antibody gold colloid forms. Dengue IgM test device contains three lines; "C" (control line), "M" (IgM test line). IgM test line is coated with anti-human IgM. When a sample is added to the device, IgM antibodies in the sample react with red particles coated with dengue proteins. As this sample/particle mixture migrates along the length of the test, the anti – dengue IgM antibody particle complex is captured by the relevant IgM test bands located in the test device window causing a pale to dark red band to form at the IgM region of the test device window. The intensity of the test bands in the respective device will vary depending upon the amount of antigen/ antibody present in the sample. The appearance of any pink/ red colour in a specific test region should be considered as positive for that particular antigen and/or antibody type (IgM). A red procedural control line should always develop in the test device window to indicate that the test has been performed properly. Consequently the samples were checked with IgM capture ELISA by national institute of virology, Pune as the reference standard. Which (1) require than technician time. The procedure starting with the coating of anti IgM was performed according to protocol provided. The intensity of colour/optical density (OD) is monitored at 450 μm. the OD values are directly proportional to the amount of virus specific IgM antibodies present in the sample. If OD value of sample tested exceeds OD of negative control by a factor 4 (sample OD 7, negative OD × 4) the sample should be considered as "Positive".

RESULTS

A total 280 dengue suspect cases from the study subjects with 78 sample testing reactive for primary dengue infection, which were tested by rapid NS1 STRIP (Table no 1).

Table 1: Table shows Comparison of Diagnosis of dengue patients with rapid ICT test & IgM ELISA test.

Total no. of patients	280
Rapid test (ICT)	NS1/IgM (Total) 78
IgM ELISA positive	49

Results obtained with rapid devices were verified for accuracy with IgM capture ELISA as the reference standard. Out of 78 positive cases 53 were males and 25 were female patients (Table no 2).

Table 2: Table shows number of positive cases in males and female of different age groups.

Sex/age	positive	0-12	13-20	21-40	>40
male	53	14	16	22	1
female	25	8	6	8	3

Among 78 cases 39 cases were positive for NS1 antigen alone, 21 were positive for IgM rapid test alone and 18 were positive for Both NS1 and IgM (Table no 3). Total 49 patients showed positivity for IgM ELISA test.

Table 3 : Table shows Rapid ICT test evaluation.

NS1 Ag	**IgM antibody**		**Total**
	Negative	Positive	
Negative	202	21	223
Positive	39	18	57
Total	241	39	280

DISCUSSION

In our study male were affected more than females. Out of 78 patient s 30 have co infection of Malaria and 4 were positive for Widal test (Table 4).

Table 4: Table shows mixed infection with malaria and Widal positivity.

Total dengue positive	78
Associated malaria infection	30
Associated Widal positivity	4

Table 5 : Comparison of rapid IgM rapid ICT and IgM ELISA methods.

Result	**IgM rapid ICT**	**IgM ELISA**
Negative	241	231
Positive	39	49
Total	280	280

Malaria is common with dengue because the mode of transmission is same. We did two diagnostic tests for early detection of dengue infection during the acute phases of the disease. The dengue NS1 antigen strip- the first ICT developed for NS1 detection- for all dengue virus serotypes. This rapid diagnostic test is convenient, easy to use and the results are obtained within 15 minutes for ambiguous results. In our study among 78 positive cases only 49 were positive by IgM ELISA, while 57 were positive for NS1 Ag by rapid ICT. It shows that in early infections (Fever within four days) the NS1 is useful diagnostic marker rather than IgM Antigen. The rapid test does not involve any specific laboratory equipments except micro centrifuge for serum separation. So the rapid test may prove to be useful aids in screening in clinical diagnosis of dengue infection, more so in the resource poor peripheral health setting. It can prove to be a useful tool to hasten the initiation of the first line of the management and thereby can be great help to the health care providers in the rural area.

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