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Original Article

Association of NS1 Antigen, IgM, IgG Antibodies and RT-PCR in the Diagnosis of Dengue Virus Infection

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Abstract

Background: To determine the association of ELISA based serological markersNS1 antigen, IgM, IgG antibodies and RT-PCR in the diagnosis of dengue virus infection

Methods: In this descriptive cross sectional study 420 serum samples from patients with suspicion of dengue fever were tested for detection of dengue by NS1 antigen ELISA, IgG, IgM ELISA. RT-PCR for dengue was carried out in all NS1 antigen ELISA positive cases for confirmation of dengue.

Results: Out of 420 cases , 249 cases were positive for either one of the three markers NS1, IgM,IgG. Males constituted 71.66%.Two hundred and two (48.09%) were positive for NS1 only,13 (3.09%) were positive for NS1 and IgG, 07 (1.66%) were NS1, IgM and IgG positive,16 (3.80%) were positive for IgG only ,11 (2.61%) were positive for NS1 and IgM whereas 171 (40.17%) samples were reported negative for NS1, IgM and IgG.RT-PCR was conducted on 233 NS1 positive cases out of which 80.06% cases turned out positive. Maximum number of cases belonged to DEN-2 genotype.

Conclusion: Early diagnosis helps in improved patient care, suitable treatment, prevents severe complications and helps limit the spread of the disease. RT PCR is a reliable test for the diagnosis of acute dengue fever.

Key Words: DENV: Dengue virus , Dengue Shock Syndrome, Dengue Haemorrhagic Fever,

Introduction

Dengue is a pandemic disease and approximately three billion people of tropical and subtropical region are at risk of this viral infection.[1] It has become a serious public health problem due to its increasing morbidity[2]. It is one of the most important mosquito borne viral disease of humans.[3]Dengue virus (DENV) belongs to Genus Flavivirus, classified into four serotypes (DEN 1-4) on the basis of different antigens present on it.[4] Infection with anyone of its four serotypes produces a spectrum of clinical illness ranging from asymptomatic to its most severe form like, Dengue Haemorrhagic fever (DHF) and Dengue Shock Syndrome (DSS).[5]

Patients present with non-specific symptoms such as fever, aches, fatigues that often overlap with other endemic infection, no vaccine or specific antiviral therapy has been approved to date to reduce the disease burden.[6] Patient management relies on good supportive care .Early diagnosis of the Dengue viral infection is crucial and has the potential to reduce morbidity and mortality. In general diagnosis of dengue is dependent on the phase of the infection[7].During the acute phase of infection isolation of virus from culture is considered to be the gold standard but it is laborious and time consuming. Nucleic acid detection (RT- PCR) and serology is also used to confirm infection and distinguish primary and secondary infection. [8,9,10] IgM/IgG ELISA are widely used as laboratory methods for rapid diagnosis in acute phase of the disease.[11-16]

Viraemia occurs during the first week of clinical symptoms of DF. At this time, direct detection of the virus by either viral ribonucleic acid (RNA) detection or viral antigen detection is appropriate. After the first week, the host antibody response to the virus develops and during this time the viraemia is reduced and host immunoglobulin M (IgM) levels rise after 5-7 days, followed by immunoglobulin G (IgG) levels after a few weeks. Early diagnosis of the Dengue viral infection is crucial and has the potential to reduce morbidity and mortality. [9,16] NSI antigen is the highly conserved glycoprotein essential for the viability of DENV. NS1 is common to all dengue serotypes and can be used to detect primary and secondary infection in patient serum as early as day one of illness and persists up to 9-10 days**.** Dengue virus NS 1antigen is useful for detection of early stages of dengue virus infection.[1,7,9,8]

There is no cross reactivity of dengue- NS 1 protein with those of other flavivirus.[15]Quantitative detection of NS1 may help predict the risk associated with DENV infection as high NS1 levels have been found to

correlate with dengue haemorrhagic fever (DHF).[16,17]Dengue specific antibodies IgM and IgG can be detected after 5 days and 2-4 weeks post infection respectively.Apart from dengue specific diagnostic tests, thrombocytopenia and hemoconcentration are consistent findings in DHF, there is a drop in platelet count levels below 1,000,000 per mm^3 usually found between the third and eighth day of illness.[18-21]

Patients and Methods

The present study was conducted in a tertiary care District teaching Headquarter Hospital Rawalpindi, from September 2016 to November 2016.Approval of institutional ethical committee Rawalpindi Medical University was taken prior to beginning of this study.A total of 420 serum samples were processed from suspected cases of dengue fever using NS1 ELISA kit (In Bios USA) for detection of NS1 antigen and for IgM/IgG antibodies (ELISA Vircell SPAIN) according to the manufacturer's instructions.Samples of all those cases found positive for NS1 were further confirmed for Dengue virus by RT-PCR.In the present study we evaluated various diagnostic methodologies i-e NS-1 antigen (ELISA), IgM and IgG ELISA and RT-PCR for detection of dengue virus infection in 420 patient samples and also analyzed the utility of these tests for diagnosing acute dengue cases.

Results

In this study males constituted 301 (71.66%) and females constituted 19(28.33%). Out of 420 serum samples tested, 249 (59.28%) were tested positive for either one or more of the three serological markers. Of these 249 samples, 202 (48.09%) were exclusively positive for NS1 antigen. 13(3.09%) samples were positive for both NS1 antigen and IgG. Thirteen(3.09%) samples were positive for all the three serological markers. Eleven(2.61%) were positive for NS1 and IgM markers. 16 (3.8%) samples were exclusively positive for IgG.Samples negative for all three serological markers NS1, IgG and IgM were 171(40.71%) (Table 1).

Table.1: Serological parameters for diagnosis of acute dengue fever (n=420)

Parameters	Total no.	Percentage %
$NS1^+$ only	202	48.09
$NS1^+$ and IgM^+	11	2.61
$NS1^+$and IgG^+	13	3.09
$NS1^+$+IgG^++IgM^+	7	1.66
IgG only	16	3.80
$NS1^-$, IgG^-, IgM^-	171	40.71

NS1positive cases (n=233) were confirmed for presence of dengue virus by RT-PCR.Samples were sent to Institute of Public Health Punjab.188(80.06%) were found to be positive.Out of these 188 cases 134(71.2%) were DEN-2 positive, 46 (24.46%)were DEN-3 positive,08 (4.25%) were DEN-1 positive. However no DEN-4 genotype was detected in all 188 PCR positive cases (Table 2).

Table.2: Genotypes of dengue virus confirmed through RT-PCR in NS-1 positive cases (n=233)

	DEN-1	DEN-2	DEN-3	DEN-4	Total PCR positive
Number of patients tested positive	08	134	46	0	188
Percentage of total	4.25%	71.27%	24.46%	0	80.06

Discussion

Regarding NS 1 antigen detection by ELISA and RT-PCR the results obtained in our study were comparable with other studies which proved the utility of NS-1 antigen and RT-PCR in acute phase of disease.NS-1 antigen positive 202 (48.09%) cases in our study were negative for IgM/IgG antibodies and these would have been missed in absence of NS1 testing. These patients were suffering from infection and they could transmit the virus. This highlights the importance of NS-1 antigen testing in the early phase of infection.An Indian study conducted at SGT hospital revealed overall efficiency of NS1 antigen detection 83.6% and RT-PCR 87.3%. [22]In a study by Dussartet al NS-1 antigen ELISA showed 60% sensitivity**.** Rico and Bessoff et al reported sensitivity of 65% for NS-1 13/9, sensitivity of RT-PCR was 83%.[23, 24]

Relevant observation made in this study was that the highest number of positive samples for NS-1 and RT-PCR were found between day two to four of the onset of symptoms and illness. Similar observations were made by Gurukumar et al and studies of other researchers revealed the utility of NS-1 antigen ELISA

and RT-PCR is maximum in the acute phase of illness.[25]

Very few studies in Pakistan have correlated dengue PCR with ELISA based antigen and antibody tests for diagnosis of acute dengue fever .In a study conducted at Armed Forces Institute of Pathology the sensitivity of dengue PCR was at 72% during the first four days of infection.[26] Similarly at Aga Khan university hospital a study conducted for the evaluation of two Elisa kits i-e IgG/IgM put the sensitivity of PCR at 87%.[27]

The most important benefit of laboratory testing is screening of patients suspected of dengue, to implement the most appropriate clinical management and to provide more efficient epidemiological surveillance system which helps control constant outbreaks of dengue feared to result in a higher incidence of secondary infection which are positively correlated with a higher risk of DSS. [17]

The main factors influencing the diagnosis were the type of Infection primary or secondary serotype of virus, geographical origin of the sample and how early the samples were collected.

Early diagnosis allows improved patient care, enables suitable treatment to avoid severe complications and helps limit the spread of the disease, tests like NS1, RT-PCR and IgM/IgG are essential for clinical management, surveillance and containment of the disease.Early diagnosis of dengue infections facilitates in prompt management of disease and hence can circumvent catastrophic outcomes. When DEN-PCR or NS1 antigen diagnosis is combined with IgM testing, the overall rates of diagnosis for dengue were > 90%. Rapid diagnosis of DENV in the early phase becomes important to distinguish from other haemorrhagic fever, such as Congo-Crimean Haemorrhagic Fever Virus (CCHF), which is important in a region endemic for both dengue and CCHF viruses. There are additional haemorrhagic fever viruses present in Pakistan, such as Chikungunya and West Nile viruses, hence these should be considered in cases where results for DENV are found to be negative. [28-31]

Conclusion

Early diagnosis helps in improved patient care, suitable treatment, prevents severe complications and helps limit the spread of the disease. RT PCR is one of the most reliable tests for diagnosis of acute dengue fever.

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