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Microbiology

A Preliminary Evaluation of Immunoassays for Detection of NS1 Antigen and IgM Antibody in Early Diagnosis of Dengue Infection among Febrile Patients

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Abstract: Early diagnosis of dengue virus infections is essential for the timely management of dengue infections. Aim: The aim of the present study is to compare results of enzyme linked immunesorbent assays [ELISA] of dengue nonstructural protein 1 [NS1]) and IgM antibody and to prove that use of NS1 with IgM ELISA tests improves the laboratory diagnosis of dengue. Settings& Design: Present cross-sectional study was conducted at a Govt. Tertiary Care Teaching Hospital, Hyderabad. One hundred seventy-four serum samples were screened from clinically suspected cases of dengue fever of one week or less duration reporting at the Tertiary Care Center, Hyderabad from August 2017 to January 2018. All 174 samples were subjected to NS1 antigen early ELISA and IgM capture ELISA. Results: 66 samples (37.93%) gave positive reaction either in NS1 Ag detection test or in IgM antibody ELISA.Twenty-two patients showed positive for NS1 antigen (12.64%) and fifty-four patients (31.03%) showed positive IgM ELISA and both the tests were positive in ten patients (5.75%). Conclusion: Dengue NS1 antigen is useful for rapid and accurate diagnosis in acute phase of illness. Combination of dengue NS1 antigen and IgM ELISA on single sample can improve the diagnosis of dengue without the requirement of paired sera.

Keywords: NS1 Antigen, ELISA, dengue infection, dengue IgM antibody, acute febrile illness, thrombocytopenia

INTRODUCTION

The incidence of dengue is increasing dramatically around the world in recent decades. The actual numbers of dengue cases are under reported and many cases are also misclassified.

One recent study indicates 390 million dengue infections per year, of which 96 million (67–136 million) manifest clinically. Another study, of the prevalence of dengue, estimates that 3.9 billion people, in 128 countries, are at risk of infection with dengue viruses. The number of cases reported increased from 2.2 million in 2010 to 3.2 million in 2015. Although the full global burden of the disease is uncertain, the initiation of activities to record all dengue cases partly explains the sharp increase in the number of cases reported in recent years [1]. With the escalating incidence of dengue infections and the absence of vaccines for the prevention of this disease, early diagnosis of dengue virus infections in patients is needed. It is challenging for us to control the infection as it requires not only effective control of vectors but also rapid, accurate, and early diagnosis. Early diagnosis can assist in patient management by directing clinical attention to the appearance of major warning signs of severe or life-threatening complication, e.g., rapid rise of haematocrit, poor peripheral perfusion. Early diagnosis also helps in preventing unnecessary, expensive usage of antibiotics and it provides important data on the epidemiology and health burden of dengue [2].

Laboratory methods like viral isolation, detection of genomic RNA, antigen and antibody are available to diagnose dengue fever. The most commonly used methods are detection of NS1 antigen, a highly conserved glycoprotein produced in both membrane associated and secretion forms, is abundant in the patient's serum during early stage of infection, immunoglobulin M (IgM) and immunoglobulin G (IgG) by enzyme linked immunesorbent assay (ELISA). IgM indicates acute infection and it can be detected only after the end of viremic phase. In contrast to IgM, NS1 Ag can be detected before the formation of antibodies. It is detectable in blood from the 1st day after the onset of fever up to day 9. It is

still detectable even when viral RNA is negative by reverse transcription polymerase chain reaction (RTPCR) [2].

MATERIALS AND METHODS

One hundred seventy four serum samples were screened from clinically suspected cases of dengue fever of 1 week or less duration, presenting with two or more of the sign and symptoms of fever with temperature more than 37.5°C/99.5°F, headache, joint pains, retro orbital pain, myalgia, rash&haemorrhagic manifestation, thrombo-cytopenia & positive tourniquet test who were reporting at the Tertiary Care level teaching hospital, in Hyderabad from August 2017 to January 2018. Permission was obtained from the institutional ethics committee and informed consent was taken from the participating patients.Patients positive for malaria after peripheral smear examination were excluded from the study. No healthy controls were included in the study as it has been amply proved that NS1 positivity is negligible in this group. All 174 samples were tested for NS1 antigen and IgM antibody by capture ELISA.

Sample Collection: Patients were explained about collection of 5ml of blood by venepuncture and the purpose, importance and benefit of getting tested for Dengue infections.5ml of venous blood was collected under aseptic precautions in vacutainers with no additives and kept at room temperature for 30min to clot. Serum was separated by centrifugation at 3000rpm for 5min and transferred to plastic aliquots. Each serum sample was distributed in three different aliquots, 2 were used for tests and a third preserved as a stock sample. The aliquots were labelled with appropriate information of patient and were stored at -20° C until tested. Serum samples from subjects were tested using Pan Bio Dengue Early ELISA kit (Inverness Medical Innovations, Australia) for detection of NS1 antigen. All the samples were tested for the presence of dengue specific IgM antibodies using MAC ELISA, developed and commercialized by the National Institute of Virology, Pune, and recommended by the National Vector Borne Disease Control Programme. Tests were done and results were read as per the literature provided. From ethylene diamine tetraacetic acid blood samples, platelet count was done and interpreted as normal, when the count was between 150,000 and 450,000 and thrombocytopenia, when the count was <100,000/cumm (WHO cut off for platelet count for DHF).

Fig-1: Panbio ELISA test kit and ELISA plate showing positive and negative results

RESULTS

174 patients were tested for dengue NS1 antigen and IgM antibody. 66 [37.93%] patients were tested positive for either NS1 antigen or IgM antibody. Patients were aged between 13 years and 65 years. Out of 97 male patients tested, 43 [44.32%] were positive for dengue infection. Out of 77 female patients tested, 23 [29.87%] were positive for dengue. Males of 11-40 years age group were more affected. (Table 1)

Table 1: Age and sex distribution of dengue positive cases

S:NO	Age group	Male(n=97)	Female(n=77)	Total
1	11 - 20	16	11	27 (46.55%)
2	21 – 30	13	6	19 (32.75%)
3	31 – 40	10	4	14 (48.27%)
4	41 – 50	3	1	4(26.66%)
5	51 – 60	1	1	2(22.22%)
6	61 – 70	0	0	0(0%)
Total		43(44.32%)	23(29.87%)	66(37.93%)

Table-2: Dengue positive cases in relation to duration of fever

S:NO	Fever: no of days	No. of samples	IgM Ab positive	NS1 Ag positive	Both IgM&NS1 positive
1	1-3	72	6(8.33%)	11 (13.75)	2 (2.77%)
2	4-5	66	29(43.93%)	7 (12.5%)	4 (6.06%)
3	6-7	36	19 (52.77%)	4(10.5%)	4 (11.11%)
		174	54 (31.03%)	22 (12.64%)	10(5.74%)

Table-3: Comparison of platelet counts of IgM antibody positive patients and NS1antigen positive patients

PLATELET COUNT	IgM positive	NS1 positive
More than 1 lakh	11(20.37%)	7(31.81%)
Less than 1 lakh	43(79.62%)	15(68.18%)
	54 (100%)	22 (100%)

Table-4: Seropositive cases by testing with different parameters for dengue infection

Tests done(n=174)	Number of positive cases	Percentage
IgM antibody	54	31.03%
NS1 antigen	22	12.64%

NS1 antigen was detected in 12.64% of the cases tested. IgM antibody was detected in 31.03%.NS1 antigen was detected more in patients with 1-3days of fever gradually falling from 13.7% to 12.5% and 10.5% by 7 days. Half of NS1 positive cases were detected in first 3 days of fever. IgM antibody was detected more after 4th day of fever (Table 2). Thrombocytopenia was seen in 68% of NS1 Ag positive cases and 79.62% of IgM positive cases (Table 3).

DISCUSSION

In the present study 37.93% patients were found to be positive for either NS1 antigen or IgM antibody. Similar seropositivity was observed by Panwala *et al*. (23.3%) [2], Palanivel *et al*. (38%)[3] & Bhattacharya *et al*. (38.3%) [4]. In a study conducted at the same centre by Nanditha A in 2015 the seroprevalence was 36% [5]. In the present study patients aged between 11-40 years were most affected. Males of working age group were more affected. Involvement of younger age group with increasing frequency in epidemics is indicator of higher incidence of infection [3].

NS1 antigen was detected in 12.64% of the cases tested. The low sensitivity obtained in this study may be linked to poor reporting for this variable (date of testing) [6]. IgM antibody was detected in 31.03%, Kulkarni *et al* observed NS1 antigen in 29.68% of cases&IgM antibody in 50.3%. [7]. Datta *et al*. reported IgM positivity 39.1% which is similar to our IgM detection rate 31.03%. [8]

50% of NS1 positive cases were in the first 3 days of fever. 82% of NS1 antigen positive cases were detected in first 5 days of fever. 89.4% cases gave positive reaction in NS1 Ag detection test at less than 5 days of fever in a study by Panwala *et al*. [2]. NS1 antigen declines usually after day five [6]. But it was detected in few patients at day seven also.89% of IgM positive cases were identified between 4-7 days of fever (Table2). More than 90% of patients are IgM positive by the 4th day of illness in a study by Wang *et al*. [9].

According to WHO NS1 antigen can be detected up to 9 days after the onset of illness. IgM antibodies are detectable in 50% of patients by days 3 to 5 after the onset of illness, increasing to 80% by day 5 and 99% by day 10. IgM levels peak about 2 weeks after the onset of symptoms and then decline [10].

Demonstration of dengue specific IgM/IgG is the mainstay for the diagnosis of dengue infection since a long time. The antibodies begin to appear on the 5th day of fever in primary infection. Sometimes, IgM/IgG antibodies cannot be detected before the 3rd day of fever in secondary DI. Therefore, there is some lag period both in primary and in secondary dengue when test will give negative results in antibodies specific tests. The NS1 Ag is available for diagnosis of DI from day 1 of fever both in primary and secondary infections. It is stated that 47% cases would miss if NS1 Ag test had not included in the testing panel. In the endemic areas, confirmation of DI mainly depends upon the rising titre in paired sera. However, repeat testing for the same infection when first sample was negative or for confirmation of DI is almost not possible in routine clinical practice. When NS1 Ag gives positive results in first 4 days of illness, there is no need of repeat testing as NS1 is highly specific marker for the diagnosis of DI [2].This study has been carried out at a tertiary care teaching hospital. It is worth mentioning here that most tertiary care teaching hospitals lack in viral culture setup. Therefore, applying gold standard tests in studies related to viral infections is out of reach of these centres [7].Virus isolation is carried out only by reference laboratories and is a time-consuming and expensive technique. The use of dengue RT-PCR is now well documented. However, its use in most laboratories is currently

difficult largely due to the stringent requirements concerning storage temperature, transportation, time between collection and extraction, and laboratory workflow. These two techniques are therefore largely restricted to surveillance systems and research [11].

Thrombocytopenia was seen in 68% of NS1 Ag positive cases and 79.62% of IgM positive cases (Table3). Panwala *et al*. observed thrombocytopenia in 63% NS1 positive cases &77.3% of IgM positive cases [2]. Palanivel *et al.* observed 49% of thrombocytopenia in NS1 positive cases&78% in IgM positive cases [3].

Table-5: Distribution of seropositive cases according to their duration of fever

Duration of fever	IgM and NS1 both positive (%)	IgM negative, NS1 positive (%)	IgM positive, NS1 negative (%)
1-3 days(n-72)	2.77%	12.5%	5.55%
4-5 days(n-66)	6.06%	4.5%	37.8%
6-7 days(n-36)	11.1%	0	41.66%
Total 174	5.74%	6.89%	25.28%

Table-6: Comparison of diagnostic tests

NS1 Antigen	IgM antibody negative	IgM antibody positive
Negative	108	44
Positive	12	10

For a diagnosis of a confirmed dengue, the virus should be identified by isolation or there should be a 4-foldrise in antibody titre. Isolation of viruses cans take 7 to 10days. The choice of a test, therefore depends on the availability of facilities, human resources and also the time of sampling [9]. NS1 Antigen is highly conserved for all dengue serotypes, circulating high levels during first few days of illness. There is no cross reaction of dengue NS1 protein with those of other related flaviviruses as it noticed in IgM ELISA. The dengue NS1 antigen was not found in patients with Japanese encephalitis virus or yellow fever virus infections thereby implying that there is no cross reaction of dengue NS1 protein with those of other related flaviviruses[12]. NS1 antigen detection was more relevant during first few days of fever (Table 2) as evidenced by 82% of NS1 positive cases being diagnosed in the first 5 days of fever. 89% of IgM positive cases were identified between 4-7 days of fever (Table2). But the IgM antibody may be due to infection 3 months earlier [9].

Dengue NS1 Antigen ELISA has a sensitivity between 76.76% and 88.7%[11] and a specificity around 98.31%[9]. In present study Pan bio Early Elisa kit was used which has a sensitivity of76% &specificity of 98.4% [5]. NS1 Ag ELISA is useful, sensitive, and specific for the diagnosis of dengue virus infection. However, caution is needed in using it as a single assay in any laboratory, as detection in samples that contained the virus was only about 81.97% [9]. The detection rate of DI increased when both the assays were used together on a single sample [7]. From table 5 & 6 it is evident that NS1 antigen test could detect 12 cases (6.89%) which were not detected by IgM antibody test. IgM antibody test detected 44 cases (25.2%) of DI which were not positive for NS1 antigen. A diagnostic strategy combining the dengue NS1 Antigen test and Immunoglobulin M capture enzyme-linked immunosorbent assay for early diagnosis increases sensitivity.

CONCLUSION

This study affirms that in comparison to IgM ELISA, NS1 antigen assay is an effective tool for diagnosis of dengue infection especially within first four days of clinical symptoms. Early detection of DHF by NSI assay helps in early diagnosis, management and prevention of complications. Combination of dengue NS1 antigen ELISA and IgM antibody ELISA on single sample can improve the diagnosis of dengue and helps in controlling the dengue infections in situations where paired sera are not available.

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