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EVALUATION OF NS1 ANTIGEN-CAPTURE ELISA AND IgM ANTIBODY CAPTURE ELISA IN ACUTE CASES OF DENGUE IN RURAL POPULATION IN A TERTIARY CARE TEACHING HOSPITAL IN WESTERN UTTAR PRADESH INDIA

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Key words:

Dengue, NS1 Antigen, IgM Antibody, dengue serodiagnosis, MAC- ELISA

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ABSTRACT: Background: Dengue is a major public health problem in resource-limited countries like India. There has always been a quest for best serological test for prompt and accurate diagnosis and whether single test or a combination be used. Enzyme immunoassays based on the detection of dengue virus non-structural protein 1 (NS1), and IgM antibody capture are available with different sensitivities and specificities observed in various settings. **Aim:** Diagnostic efficacies of two enzyme immunoassays, i.e. non-structural 1 (NS1) antigen-capture ELISA against IgM antibody capture ELISA to confirm cases of dengue infection in single acute serum samples have been evaluated and each test compared against the combination of the two. **Material and methods:** NS1 antigen (QUALISA Dengue NS1) and IgM capture ELISA (Calbiotech Dengue Virus IgM ELISA) were performed on 254 single acute serum and interpreted as per the manufacturer's instructions. **Results:** The NS1 antigen detection and IgM antibody ELISA gave 25.59% and 17.3% positivity rates, respectively. The combination of two assays increased the overall detection rates to 30.7%. Noteworthy here is, that detection rate improved significantly to 55.72% (McNemar, $p < 0.05$) when two assays were combined together as compared to each test alone. The two tests together detected positive 34 and 13 samples more than those detected by IgM capture and NS1 Antigen capture ELISA alone respectively. **Conclusion:** The detection rate increases significantly when both NS1 and IgM tests are combined and this can prove helpful in diagnosis and better management of the cases.

INTRODUCTION: Dengue is an arboviral infection transmitted by *Aedes aegyptii* and *Aedes albopictus* mosquitoes. Globally it has emerged as a serious life threatening public health burden [1]. It affects more than 2.5 billion people annually and 975 million belonging to tropical and subtropical countries in Southeast Asia, the Pacific and the Americas with Africa bearing the major brunt of the disease amounting to 900 million cases annually [2-4].

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Kolkata in India was the first to witness the epidemic (1963), but many more regions from the country reported the same in different time frames, Visakhapatnam (1964), Vellore (1968), Ajmer (1969), Kanpur (1969), Jalore (1985), Chandigarh (2002), Mumbai (2004), Ludhiana (2007), New Delhi (1996, 2003, 2006, 2010), Chennai (2006-2008) and Kerala (2008) [3, 5, 6].

Millions of cases appear every year and nearly half-a-million people develop dengue hemorrhagic fever (DHF)/dengue shock syndrome (DSS), with a 2.5 % of case- fatality rate [7]. Vaccine development is major challenge due to the fact that DHF/DSS is associated with secondary infection and that the ideal vaccine should induce robust immune response against all four serotypes [8]. The dramatic

increase in incidence, distribution and severity of DHF and DSS in last few decades may be due to unavailability of any licensed vaccine formulation to control the disease [9, 10]. The IgM capture ELISA or MAC-ELISA is based on detecting dengue virus specific IgM antibody. The assay shows the sensitivity and specificity of 90% and 98% respectively when compared to haemagglutination inhibition in sera collected after five days of fever [8]. Any of the routinely used tests like, viral nucleic acid detection by Reverse Transcription Polymerase Chain Reaction (RT-PCR), dengue NS-1 protein antigen or anti-dengue IgM/IgG antibodies, can be used to diagnose a case of dengue, however, IgM capture ELISA has always been the choice due to its cost effectiveness, availability and ease of handling.

IgM Capture ELISA is more suitable after five days of infection (late infection) and for the diagnosis of early infection, RT-PCR, virus culture or NS-1 antigen detection is the most reliable and sensitive assay, but virus culture is a less practical approach. Although RT-PCR is very sensitive and specific, it requires advance infrastructure and expertise, hence immunoassays remain the most widely used tests [7, 9, 11]. Combined use of NS1 antigen and IgM antibody ELISAs have been proved ideal in several studies to confirm dengue during both early and late infection[12]. Many studies have evaluated the use of NS1 antigen ELISA against IgM antibody ELISA using single acute serum samples and concluded that combined use of antigen and antibody capture immunoassays are most effective in prompt diagnosis and treatment [13, 14].

Here, in this study, we have performed both types of immunoassays, NS1 and IgM, in the samples received in our laboratory from various clinical departments of this Institute and the results of the combined tests have been compared individually with each test separately.

MATERIAL AND METHODS:

Samples: Serum samples of all the 254 cases clinically suspected of dengue infection presenting to the different clinical outpatient departments of this Institute were collected and received at Microbiology Department. After collection, samples were allowed to clot at room temperature and then serum were separated and stored at -80^{0}C refrigerator.

Dengue NS1 antigen detection:

All the sera were subjected to QUALISA NS1 Dengue (Qualpro Diagnostics, Goa, India) for detecting NS1 antigen. The test is based on sandwich format with monoclonal anti dengue NS1 antibodies coated microtiter plate enzyme immunoassay for the detection of dengue virus NS1 antigen in human serum and it was performed as per the manufacturer's instructions.

Anti-dengue IgM antibody detection:

All these sera samples were also subjected to serological assay of anti-dengue IgM antibody present in samples and it was carried out using a commercial IgM-capture ELISA kit (Calbiotech Inc. Spring Valley, CA). The test is based on the indirect ELISA format with microtiter plate wells coated with crude dengue antigen. The assay was performed, and the results read and interpreted as per the manufacturer's instructions.

Statistical analysis:

Statistical analysis was calculated using SPSS statistical package (SPSS, Chicago, IL) and McNemar test. Samples giving equivocal or indeterminate results were regarded as negative for the analysis.

RESULTS:

All the 254 samples were subjected to IgM antibody capture ELISA, which reported 44 samples positive, detection rate 17.32%, and rest 210 were negative for the same. On the other hand NS1 antigen capture ELISA was positive in 65 samples, detection rate of 25.59%, out of the total 254 (**Table. 1**). The combination of the two ELISAs detected 78 samples positive, detection rate of 30.7%, and that was 34 samples more than those detected by IgM detection ELISA and, 13 samples more than the 65 already detected by antigen detection ELISA (**Table 2** and **3**). Comparing the results, combination of the two ELISAs detected 55.72% and 18.11% more than those detected by IgM antibody capture ELISA and NS1 antigen capture ELISA alone, respectively.

Interestingly, detection rate improved significantly to 55.72 % (McNemar, $p< 0.05$) when both tests were applied simultaneously to the dengue suspected serum samples and the effect of combined test were compared with each individual test (**Table 2** and **3**). Interesting here to note is, that 65 serum samples tested positive by NS1 Ag ELISA, included 31 samples that were positive for IgM antibody and 34 samples positive for NS1 antigen. Of the 44 samples tested positive by dengue IgM capture ELISA, 31 samples were also positive for IgM antibody as well as NS1 antigen, while only 13 samples were positive for IgM antibody alone.

TABLE 1: COMPARISON OF RESULTS OF 254 SERA TESTED USING THE NS1 ANTIGEN CAPTURE ELISA AND IgM ANTIBODY CAPTURE ELISA

		NS1 Antigen Capture ELISA (QUALISA Tulip)		Total
		Negative	Positive	
IGM capture ELISA	Negative	176 (69.29%)	34 (13.38%)	210 (82.67%)
	Positive	13 (5.11%)	31 (12.20%)	44 (17.32%)
Total		189 (74.40%)	65 (25.59%)	254 (100%)

TABLE 2: COMPARATIVE DETECTION RATES OF NS1 ANTIGEN CAPTURE ELISA VERSUS COMBINED TESTS (NS1+IgM ELISA)

		Combined test results (NS1+IgM)		Total
		Negative	Positive	
NS1 Antigen	Negative	176 (69.29%)	13 (5.11%)	189 (74.40%)
Capture ELISA	Positive	0 (0%)	65 (25.59%)	65 (25.59%)
Total		176 (69.29%)	78 (30.7%)	254 (100%)

TABLE 3: COMPARATIVE DETECTION RATES OF IgM CAPTURE ELISA VERSUS COMBINED TESTS (NS1+IgM ELISA

		Combined test results (NS1+IgM)		Total
		Negative	Positive	
IGM capture ELISA	Negative	176 (69.29%)	34 (13.38%)	210 (82.67%)
	Positive	0 (0%)	44 (17.32%)	44 (17.32%)
Total		176 (69.29%)	78 (30.7%)	254 (100%)

DISCUSSION: There has been annual surge in the cases of dengue in India, and this needs to be addressed timely to control its spread and to effectively manage acute cases of DHF and DSS in the country. Assays based on the detection of IgM antibody or NS1 Antigen, are two most common tests used in most of the laboratories worldwide. Although molecular tests like RT-PCR (Reverse Transcription Polymerase Chain Reaction) are available but restricted only to higher centers in a country like India. Thus, it is important to understand the significance and diagnostic efficacy of serological tests like IgM capture ELISA and NS1 antigen capture ELISA in cases of dengue. The IgM capture ELISA is most commonly used in a resource limited country like India due to its low cost and ease of handling. But here it is important to understand that, NS1 antigen detection assay has an advantage over IgM detection that it can diagnose a case of dengue while the latter cannot, because IgM and IgG antibodies remain detectable for months after the clinical illness and hence test results obtained from single sera are only suggestive of infection [12]. To confirm a case of acute dengue infection by serology, IgM seroconversion or a fourfold increase of IgG antibody titer in paired sera must be demonstrated[15].

This study compares the two types of ELISAs based on two biomarkers i.e. NS1 antigen and IgM antibody in cases of dengue presenting to this Hospital. NS1 antigen is detectable by most of the commercial kits in first 7 to 9 days of infection while IgM antibodies are detectable only after 4 to 5 days of infection, the reason why NS1 antigen capture ELISA could detect more cases compared to IgM capture ELISA alone [15-17]. It has been demonstrated in many studies that the diagnostic efficacy of the combination of assays is higher than the individual test alone [14, 16, 18]. It is clear that ELISAs based on a single biomarker, NS1 antigen or IgM antibody have limitations when such tests are used individually, however, their combination

yields acceptably high levels of accuracy in diagnosing acute cases [19]. The results presented in this study also support the findings of the previous studies, which favor the use of combination of tests and it is clear that detection of single analyte NS1 or IgM is not sufficient to provide diagnostic accuracy in cases of dengue. Here, in this study we have reported overall increase in detection rate of cases to 65.38% by combination of two ELISAs as compared to 25.59% and 17.3% by NS1 antigen capture ELISA and IgM antibody detection ELISA alone respectively.

This suggests that NS1 antigen ELISA is perhaps a more sensitive and suitable test for the diagnosis of dengue cases in the laboratory. We have observed here, that the combined use of NS1 antigen/IgM antibody detection test yielded a significantly higher detection rate of dengue ($p<0.05$) than individual test, which is in agreement with other previous studies [15, 20].

Although NS1 ELISA along with IgM ELISA would double the cost, it significantly adds to detection rate and is esp. useful in diagnosing early cases of dengue.

CONCLUSION: Authors conclude here suggesting that although NS1 antigen ELISA is very useful and specific tool in diagnosing cases of acute dengue infection but when combined with IgM antibody ELISA, it can significantly improve diagnostic efficacy in dengue infection. This can definitely help resource-limited countries like India which experiences outbreak annually and it is a seasonal trend.

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