



COMPARISON OF ELISA AND NS1 ANTIGEN CARD TESTING RESULTS FOR CLINICALLY SUSPECTED DENGUE FEVER CASES IN A TERTIARY CARE FACILITY, BUNDI, RAJASTHAN

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Introduction

The four different serotypes of the Flaviviridae family's positive stranded RNA (ribo nucleic acid) virus that causes dengue (Dengue Virus 1–4) are closely linked antigenically. Only temporary and partial protection against recurrent infection by the other three serotypes is provided by recovery from one kind of infection, but lifetime immunity against that serotype is granted. Sequential infection has been proposed as a risk factor for more severe illness, including DHF.(1,2,3,4) From 505,430 cases in 2000 to over 2.4 million in 2010 and 4.2 million in 2019, the number of dengue cases reported to WHO has more than eight times grown in the past 20 years.(5)

While the mortality rate for untreated instances of DHF/dengue shock syndrome (DSS) might reach 20%, the mortality rate for treated cases is only 1%. The virus can be found in serum, plasma, circulating blood cells, and other organs for four to five days after the sickness starts. For this reason, many labs are now using the Non-Structural 1 Dengue Antigen (NS1 Ag) detection test, and the results are encouraging. The development of dengue vaccines has been underway for several decades, but has been impeded by the disease's complicated pathophysiology, the requirement to control four virus serotypes at once, and vaccine scientists' lack of funding. To prevent the ADE (Antibody dependent enhancement) phenomena, the vaccination should offer protective protection to all four serotypes. (6) Before these vaccinations may be used widely and safely on the susceptible populations, many obstacles (including practical, logistical, and scientific) must be overcome when they are produced. (7)

Additionally, there are no antiviral medications that work on the market. For severe types of the condition, the only known treatment is supportive care, such as fluid replacement.(8). Vector control is the only way to stop dengue fever (DF) from spreading if there is no specific therapy or vaccine. Continuous sero-epidemiological surveillance is necessary for the prompt development and execution of an efficient dengue control program, since effective control and preventative programs rely on enhanced surveillance data.(9)

Early laboratory diagnosis of dengue virus infection is crucial because it can lead to better prognosis and early intervention. This is especially true if the disease is identified before the defervescent phase, when there is a higher risk of DHF from plasma leakage and the evolution to shock. Serological testing is frequently used for this purpose.

Materials and Methods

This study was conducted over ten months, from February 2024 to November 2024, in a tertiary care hospital in Bundi. It includes ward-admitted patients with a clinical suspicion of dengue. Every suspicious case yielded five millilitres of blood. According to the manufacturer's instructions, serum

was separated and subjected to an immunochromatography (ICT) based RDT lateral flow assay (Advantage Dengue NS1 Ag & Ab Combi Card test, Jai Mitra & co. Ltd., New Delhi) to check for the presence of IgM and IgG antibodies as well as NS1 Ag.

IgM (PanBio dengue IgM capture ELISA test) and IgG antibodies were detected in all serum samples (PanBio dengue IgG capture ELISA test). The ELISA test was conducted in accordance with the manufacturer's instructions.

Results

The fast immunochromatography card test was used to check for the presence of NS1 antigen and IgM and IgG antibodies in 521 clinically suspected dengue cases.

Additionally, capture ELISA for IgM and IgG antibodies was performed on them. Using these techniques, 228 cases were verified to be dengue positive.

Table 1: Total no. of serologically positive cases by rapid, ELISA and by both the tests

RAPID	ELISA	ELISA + RAPID
222	171	228

A total of 123 cases were positive for IgM by ELISA as compared to 117 cases by rapid card test.

Table 2: Serologically positive cases diagnosed by rapid tests:

NS1 Ag	61	27.5%
Antibody	161	72.5%
IgM	72	32.4%
IgG	40	18.0%
IgM + IgG	17	7.7%
NS1 +IgM	25	11.3%
NS1+IgG	04	1.8%
NS1 +IgM+IgG	03	1.3%
Total Positive sample	222	100%

IgM positivity was seen in 32.4% of cases followed by NS1 positivity in 27.5% cases.

Table 3: Serologically positive cases diagnosed by ELISA test

IgM	IgG	IgM+IgG	Total Positive
99	48	24	171
57.9%	28.1%	14.0%	100%

IgM positivity rate was 57.9% by ELISA test.

Table 4: Comparison of IgM antibody by rapid card test and ELISA test

Rapid card test (IgM)	ELISA
117	123
51.3%	53.90%

(Chi square=123.9 (df=1), $p < 0.001$, is significant)

Table 5: Comparison of IgG antibody by rapid card test and ELISA test

Rapid card test (IgG)	ELISA
64	72
28.10%	31.60%

(Chi square=159.2 (df=1), $p < 0.001$, is significant)

A total of 72 cases were positive for IgG by ELISA as compared to 64 cases by rapid card test

Discussion

222 samples in all were positive on the card test. Only 61 (27.5%) of these 222 positive samples tested positive for the NS1 antigen. The current study's findings agreed with Kulkarni et al. (2011)'s findings (30%). In 23.3% and 16% of the cases, respectively, Datta (2010) (10) and Srivastava (2011) (11) have demonstrated NS1 positive. Given the extremely high specificity of NS1, it may be said that 27.5% of the cases in the current study would have gone unnoticed if NS1 antigen detection had not been performed. Furthermore, NS1 was positive in IgM + IgG (1.3%), IgM (11.3%), and IgG (1.8%) combinations. In the current study, 14.4% of cases had positive NS1 in combination (IgM, IgG, and IgM+IgG). In 11.3% of NS1 Ag + IgM and NS1 Ag + IgG combo cases, Kulkarni et al.(9) had demonstrated positive. A recent study by Tricou et al. (2010) assessed the effectiveness of two additional commercial NS1 fast tests and found that combining NS1 and IgM/IgG testing improved test sensitivity overall without lowering specificity. Forty individuals (18%) tested positive for IgG only, and 72 (32.4%) tested positive for IgM exclusively. In their investigation into the relationship between platelet counts and serological markers, Kulkarni et al.7 found that 50% of the subjects had IgM positive, 3% had IgG positivity, and 6% had IgM + IgG positivity. The IgM + IgG positive rate in the current study was 7.7%. The current study found a high positivity rate for IgG only, and its results for IgM only and IgM + IgG correlated with those of Kulkarni et al.7. This is explained by the presence of an IgG antibody brought on by prior dengue illness exposure. However, even previous subclinical infections can result in detectable IgG Ab in the blood, which may last for years after the infection.

In contrast to 117 cases by quick card test, 123 cases by ELISA were positive for IgM (99 for IgM only and 24 for IgM plus IgG). Thus, the quick card test missed six cases (2.6%) of IgM positive. According to the literature, ELISA has a higher sensitivity than the fast card test. As a result, the ELISA test in this study found more instances. Similar findings were also observed in the IgG ELISA instance, where 72 patients tested positive but only 64 cases tested positive on the quick card test. Thus, the quick card test missed 8 situations (3.5%). In a study by Aikat et al.,(13) rapid tests revealed a prevalence rate of dengue infection to be 17.7% compared to 18.3% with ELISA indicating a slightly lower accuracy for rapid test results.

Conclusion

Clinical management depends heavily on an accurate and efficient diagnosis of dengue (i.e., early detection of severe cases, case confirmation, and differential diagnosis with other infectious diseases). Prior to arriving at our hospital, the majority of cases were referred from other locations and had been treated for a few days. In many cases, it was not possible to determine the exact day of fever at the time of the test. Despite this, 61 patients had positive NS1 results. Antigen detection shows promise as a practical method of infection diagnosis in the early stages of the illness. Serology is the preferred diagnostic technique towards the conclusion of the acute phase of infection. It is obvious that if the trend of emerging diseases is to be reversed, the focus must be on disease prevention. Effective vector control strategies and ongoing community engagement are essential for successful disease prevention initiatives.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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