COMPARATIVE STUDY OF RDT AND ELISA KIT FOR DIAGNOSIS OF DENGUE VIRUS INFECTION

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ABSTRACT

Dengue fever (DF) is a viral disease generally transmitted by mosquitoes of the Aedes genus: Aedes aegypti. A total of 261 samples were tested by IgM Capture ELISA and Dengue IgM/IgG RDT Kit, out of which 18.8% of dengue-positive cases were detected by IgM Capture ELISA and 15.3% by the RDT Kit. The sensitivity of the RDT Kit was found to be 73.46% in comparison to ELISA. Among the suspected cases, 67.4% were male and 32.6% were female. The age group 15–50 years had the highest dengue-positive cases (81.6%). Students had the highest number of positive cases (32.7%) in the professional group. Patients with joint pain, retro-orbital pain, and skin rash as major symptoms were diagnosed as dengue-positive by RDT and ELISA test methods. The study focused only on dengue-positive or negative cases among suspected patients, but there is a need for molecular tests such as PCR for identification of serotypes circulating among suspected cases.

Keywords: DVI, IgM Capture, ELISA, RDT, Clinical Features, PCR.

INTRODUCTION

Dengue fever is a mosquito-borne viral illness generally transmitted by *Aedes aegypti*, belonging to the family Flaviviridae. The origin of the dengue virus is believed to lie in a forest cycle involving lower primates (monkeys) and mosquitoes. The dengue virus is a single-stranded RNA virus, as shown in Figure 1, and has four serotypes: DENV-1 to DENV-4. It causes dengue fever, dengue hemorrhagic fever (DHF), and dengue shock syndrome (WHO, 2009a).

Abbreviation

DENV: Dengue Virus

RDT: Rapid Diagnostic Test

ELISA: Enzyme linked Immuno Sorbent Assay

PCR: Polymerase Chain Reaction

DVI: Dengue Virus Infection

DF: Dengue Fever

DHF: Dengue Hemorrhagic Fever

PCR: Polymerase Chain Reaction

1. Transmission

The major transmitive agent of dengue viruses (DVs) to

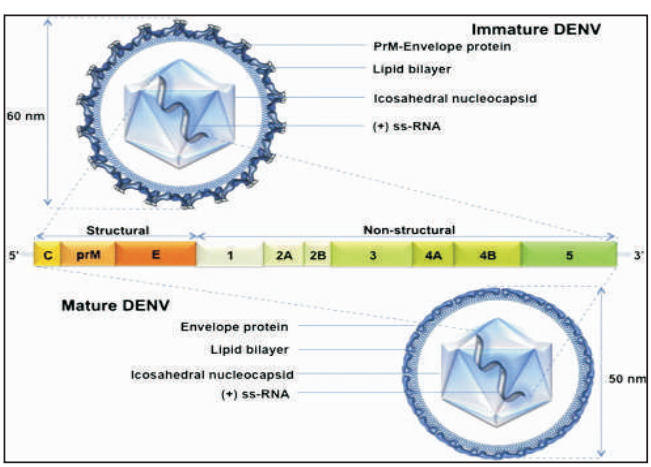

Figure 1. Morphology and Genome Structure of Dengue Virus

humans includes mosquitoes of the *Aedes genus: Aedes aegypti.* The mosquito lives in close contact with humans and lays eggs in freshwater containers and feeds on humans rather than other vertebrates (Gubler, 1989). Dengue virus infections are related to different environmental factors: weather and climate, human behaviors, the load of mosquitoes in areas, and susceptible humans (Schreiber, 2001).

1.1 Symptoms

Symptoms of Dengue fever DHF varies from high fever, skin rash, retro-orbital pain, myalgia, and low numbers of platelets to circulatory failure (WHO, 2009b). Dengue hemorrhagic fever has a higher death rate than primary dengue virus infection (Deen et al., 2006).

1.2 Diagnostic Method

Dengue virus diagnosis methods include virus isolation and their characterization (molecular methods) and detection of antibodies and antigens (serological method) (WHO, 2009b).

1.3 Dengue Virus Infection in Nepal

Dengue Virus Infection was first reported in foreigners in Nepal (Kurane et al., 2000; Pandey et al., 2004). A Larger outbreak of dengue fever occurred in 9 districts in 2006 (WHO, 2009b; Epidemiology and Disease Control Division, Department of Health Services, 2019). The presence of DENV-1, DENV-2, DENV-3 and DENV-4 serotypes in the territory of Nepal indicates the chances for the epidemic of DF/DHF in the country (Pandey et al., 2008).

In Nepal, the risk of dengue virus infection is very high, diagnosis and management of dengue is based on clinical symptoms and fewer Seroprevalence study has been conducted in Nepal. Pandey et al. (2008) highlighted a sensitive yet cost-effective test method for the diagnosis of dengue virus infection in Nepal, where molecular techniques cannot be easily adopted due to high costs and the availability of such facilities being limited to reference laboratories.

2. Objectives

- To compare the sensitivity of RDT and IgM Capture ELISA for diagnosis of dengue virus infection in suspected cases.
- To study the relation of dengue virus infection among different demographic groups.

3. Methodology

3.1 Materials

3.1.1 Equipments

- Multi ELISA Reader Model 2010 Anthos, Austria
- Vortex shaker Genie
- Oven CG
- Digital camera Canon
- Cold chamber Diversified Biotech
- Ice box Rush
- Autoclave Life
- Glassware Borosil
- ELISA Kit (Standard Diagnostic INC., Korea)
- RDT Kit (Panbio, Australia)

3.1.2 Methods

The blood sample collection was done at Narayani Sub-Regional Hospital, Bhawani Hospital and Research Centre, and Advance Hospital, Birgunj, from August 2023 to November 2023. A total of 261 serum samples were collected from suspected cases from 8 districts of the Terai region having symptoms like high fever, body ache, retro-orbital pain, and skin rashes. The patient's personal information and symptoms were collected by direct interview and recorded in the Dengue Case Details and Laboratory Investigation Form. The test was performed at Everest International Clinic and Research Center (EICRC), Kalanki, Kathmandu.

4. Ethical Clearance

Written consent was taken from all suspected patients before collecting blood samples in case details and laboratory investigation forms.

4.1 Dengue Case Details and Laboratory Investigation Form

I agree to provide blood samples in study titled "Seroepidemiological Study of Dengue Virus Infection in Parsha District of Nepal." and agree to be part of study.

Sign:

Date:

4.1.1 Sample Collection, Storage and Transport

Blood samples were collected in sterile, clean, dry test tubes from suspected cases (5 ml from adults and 3 ml from children). Serum was separated in a centrifuge at 3000 rpm for 5 minutes. The serum samples were transported to EICRC and stored at 2-8°C until tested.

5. Laboratory Test

5.1 Detection of Anti-Dengue IgM by RDT

5.1.1 Panbio Dengue Kit (Panbio, Australia)

Serum samples and RDT kit components were allowed to maintain room temperature (20-25°C) before performing the assay. The Panbio Dengue kit was placed on a dry and smooth surface, and 10 µl of serum specimen along with two drops of buffer was added into the circular sample well. Test results were recorded 15 minutes after the addition of the serum sample and buffer to the test kit.

6. Interpretation of the Result

One pink line at "C" indicates no dengue infection, while two pink lines at "C" and "M" indicate positivity for IgM antibodies to the dengue virus, as shown in Figure 2. This finding is indicative of a primary dengue infection.

6.1 Detection of Anti-Dengue IgM by IgM-Capture ELISA (Standard dignostic inc, Korea)

The ELISA kit was inserted into the strip holder. Five micro wells were labelled as controls, 2 wells as positive control, and 3 wells as negative control (N). Monoclonal antibody (MAb) and antigen were mixed (positive control), and 100 µl of diluted patient sample and positive controls were pipetted into their respective microwells of the assay plate. The plate was covered and incubated for 1 hour at 37°C.The wells were washed five times with diluted wash buffer. 100 µl microliters of diluted anti-dengue HRP conjugate solution were pipetted into the wells. The plate was covered and incubated for 1 hour at 37°C. The wells were washed five times with diluted wash buffer, and 100 µl of mixed TMB solution was pipetted into each well. The plates were incubated at room temperature (15-30°C) for 10 minutes. A blue color was developed. Then 100 µl of stop solution was pipetted into all wells and mixed well. The blue color changed to yellow. The absorbance of each well was taken within 30 minutes at a wavelength of 450 nm with a reference filter of 620 nm by using Multi ELISA Reader Model 2010 (Anthos, Austria).

7. ELISA Result Interpretation

On the basis of the cut-off value, the tests were interpreted as positive or negative. A sample was considered positive if its absorbance was greater than the cut-off value, and negative if its absorbance was less than the cut-off value, as shown in Figure 3.

Cut-off value = mean absorbance of negative controls + 0.300

7.1 Statistical Analysis

The obtained data from suspected cases were analyzed using Statistical package for social science (SPSS) software (version 16.0).

8. Results

Table 1 shows the results of the RDT and IgM Capture ELISA assays. The findings show differences in detection rates between the two methods, with ELISA identifying a higher proportion of dengue-positive cases. This highlights the

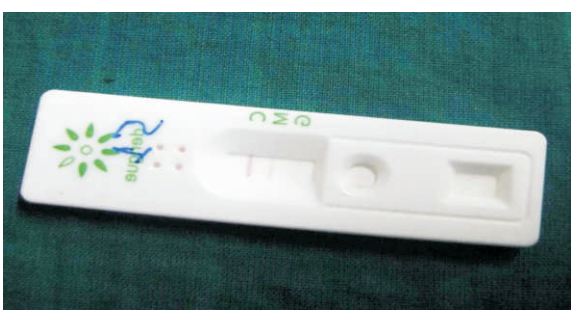

Figure 2. Test Result on RDT Kit

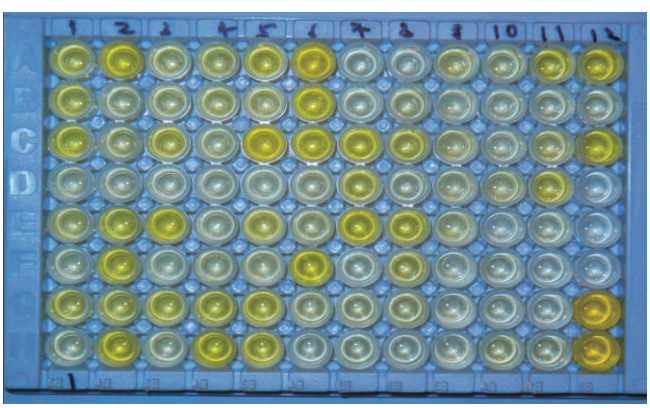

Figure. 3 Test Result on ELISA Kit

		ELISA		
RDT	Positive	36 (a)	4 (b)	40 (a+b)
	Negative	13 (c)	208 (d)	221 (c +d)
	Total	49 (a+c)	212 (b+d)	261 (a+b c+d)

Table 1. RDT and IgM Capture ELISA Assay

greater sensitivity of ELISA compared to the RDT kit in confirming dengue infection.

- *Sensitivity:* a/(a + c)*100% = 73.46%
- *Specificity:* d/(b + d)*100% = 98.1%
- Predictive value of positive test = a/(a + b)*100% = 90%
- Predictive value of negative test = d/(c + d)*100% = 94.1%
- Percentage of false negative = c/(a + c)*100% = 26.5%
- Percentage of false positive = b/(b + d)*100% = 1.9%

8.1 Age Wise IgM Positive Case

Table 2 shows the age-wise distribution of IgM-positive cases. The highest number of positive cases, 40 (81.6%), was found in the 15–50 years age group, while the lowest, 4 (8.2%) cases, was observed in participants below 15 years of age.

8.2 Sex Wise IgM Positive Cases

Table 3 shows the sex-wise distribution of IgM-positive cases. Of the total, 33 (67.4%) were males and 16 (32.6%) were females, giving a male-to-female ratio of 2.06:1.

Age	Suspected Cases	IgM Positive Cases and %	% of IgM Positive Case: n(49)	p-Value
< 15	47	4(8.2)	1.6	
15 – 50	186	40(81.6)	15.3	0.124
>50	28	5(10.2)	1.9	
	261	49(100)	18.8	

Table 2. Age Wise IgM Positive Case

Sex	Suspected Cases	IgM Positive Case Cases and %	% of IgM positive case: n(49)	p-Value
Male	134	33(67.4)	12.7	
Female	127	16(32.6)	6.1	0.013*
Total	261	49(100)	18.8	

* Statistically Significant

Table 3. Sex Wise IgM Positive Cases

8.3 IgM Positive Among Profession Group

Table 4 shows the distribution of IgM-positive cases among different professional groups. Students accounted for the highest proportion of positive cases (6.2%).

8.4 Symptoms Among IgM Positive Patients

Table 5 shows the symptoms observed in anti-dengue IgM-positive cases (n = 49). Fever was present in all patients 49 (100%), followed by headache 32 (65.3%), retro-orbital pain 22 (44.9%), muscular pain 21 (42.8%), and joint pain 19 (33.9%). These were identified as the major symptoms among IgM-positive cases.

9. Discussion

IgM positivity by ELISA and RDT was 18.8% and 15.38%, respectively. The sensitivity of the RDT kit was found to be 73.4%. The result is in close agreement with previous studies that reported 70% and 69.2%, respectively, but seropositivity was not in agreement with findings of 38.17% in 2012 and 30% in 2009 (Pun et al., 2012; Fry et al., 2011; Sah et al., 2009). The sensitivity of the RDT kit is low, and the method should not be used as the sole

Profession	Suspected Cases	IgM Positive Cases	% of Positive Cases	p-Value
Farmer	21	4	1.6	
Business	45	6	2.3	
Student	90	16	6.2	
Housewife	37	8	3	0.899
Officer	31	7	2.7	
Others	37	8	3	
Total	261	49	18.8	

Table 4. IgM Positive Among Profession Group

Symptoms	No. of Cases	% of IgM Positive Case	p-Value	Odds Ratio
Fever	49	100	_	¬_
Headache	32	65.3	0.079	1.779
Nausea	16	32.6	0.104	1.750
Vomiting	12	24.5	0.00*	22.59
Retro orbital pain	22	44.9	0.00*	7.037
Skin rash	11	22.4	0.02*	3.546
Joint pain	19	38.8	0.03*	2.723
Abdominal pain	11	22.4	0.284	1.515
Lethargy	15	30.6	0.747	1.118
Muscular pain	21	42.8	0.065	1.815
Mucosal bleeding	6	12.2	0.00*	14.651

* Statistically significant

Table 5. Symptoms in Anti-Dengue IgM Positive Cases (n=49)

technique for the diagnosis of dengue virus infection. A sociodemographic study shows that the active age group has a higher rate of infection of the dengue virus. This might be because *Aedes aegypti* is a day-biting species and lives in close association with humans. The active age group has higher activities during the day period and hence has a higher chance of getting infected by the dengue virus. Symptoms like vomiting, retro-orbital pain, joint pain, and mucosal bleeding were found to be major symptoms among dengue-positive cases. The study only focuses on dengue positive or negative among suspected cases using serological methods, but there is a need for molecular tests, like the PCR method, for the identification of serotypes circulating among suspected cases in future studies.

10. Limitations

Serological studies have a low detection rate and chances of false positive results, so the final diagnosis of dengue infection shall be confirmed by molecular tests like PCR methods.

The results of serological kits vary from manufacturer to manufacturer, so one should not totally rely on diagnosis by the RDT Kits method. Clinical symptoms shall also be considered during diagnosis of dengue virus infection.

Conclusion

Sensitivity of IgM capture ELISA was found higher in comparison to RDT Kit, so the use of IgM capture ELISA shall be used as an alternative where molecular methods are not available. The findings suggest that reliance on RDT kits alone may lead to underdiagnosis due to their lower sensitivity. Integration of ELISA in routine diagnostic practices can improve the accuracy of dengue detection and support timely patient management. Furthermore, the study highlights the importance of combining laboratory tests with clinical evaluation for a more reliable diagnosis. Future research incorporating molecular techniques such as PCR is essential to identify circulating serotypes, improve surveillance, and guide effective control measures.

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References

[1]. Deen, J. L., Harris, E., Wills, B., Balmaseda, A., Hammond, S. N., Rocha, C., & Farrar, J. J. (2006). The WHO dengue classification and case definitions: Time for a reassessment. *The Lancet,* 368(9530), 170-173.

https://doi.org/10.1016/S0140-6736(06)69006-5

[2]. Epidemiology and Disease Control Division, Department of Health Services (2019). *National Dengue Guidelines for Clinical Management, Prevention and Control.* Kathmandu, Nepal. World Health Organization. Retrieved from.

https://www.who.int/docs/default-source/nepal-documents/national-guidelines-on-prevention-management-and-control-of-dengue-in-nepal.pdf?sfvrsn=e02216fd_2

[3]. Fry, S. R., Meyer, M., Semple, M. G., Simmons, C. P., Sekaran, S. D., Huang, J. X., & Cooper, M. A. (2011). The diagnostic sensitivity of dengue rapid test assays is significantly enhanced by using a combined antigen and antibody testing approach. *PLoS Neglected Tropical Diseases,* 5(6), e1199.

https://doi.org/10.1371/journal.pntd.0001199

[4]. Gubler, D. J. (1989). *Aedes aegypti* and *Aedes aegypti*-borne disease control in the 1990s: Top down or bottom up. *American Journal of Tropical Medicine and Hygiene,* 40, 571-578.

https://doi.org/10.4269/ajtmh.1989.40.571

[5]. Kurane, I., Takasaki, T., & Yamada, K. I. (2000). Trends in flavivirus infections in Japan. *Emerging Infectious Diseases,* 6(6), 569.

https://doi.org/10.3201/eid0606.000603

[6]. Pandey, B. D., Morita, K., Khanal, S. R., Takasaki, T., Miyazaki, I., Ogawa, T., & Kurane, I. (2008). Dengue virus, Nepal. *Emerging Infectious Diseases,* 14(3), 514.

https://doi.org/10.3201/eid1403.070473

[7]. Pandey, B. D., Rai, S. K., Morita, K., & Kurane, I. (2004). First case of Dengue virus infection in Nepal. *Nepal Medical College Journal,* 6(2), 157-159.

[8]. Pun, R., Shah, Y., Gupta, G. P., Sherchand, S. P., & Pandey, B. D. (2012). Prognostic value of rapid test for diagnosis of dengue in Nepalese patients during 2010 epidemic. *Kathmandu University Medical Journal,* 10(1), 3-6.

https://doi.org/10.3126/kumj.v10i1.6905

[9]. Sah, O. P., Subedi, S., Morita, K., & Pandey, B. D. (2009). Serodiagnosis of dengue by particle agglutination assay. *Journal of Nepal Health Research Council,* 7(1), 29-32.

[10]. Schreiber, K. V. (2001). An investigation of relationships between climate and dengue using a water budgeting technique. *International Journal of Biometeorology,* 45(2), 81-89.

https://doi.org/10.1007/s004840100090

[11]. Singh, S. K., & Dutta, K. (2025). Seroprevalence study of dengue virus infection. *International Journal of Current Microbiology and Applied Sciences* 14(02): 193-205.

https://.doi.org/10.25259/GJHSR_22_2025

[12]. WHO. (2009). Dengue and Dengue Hemorrhagic Fever. Retrieved from.

https://www.who.int/news/item/15-05-2009-dengue-and-dengue-haemorrhagic-fever-(dhf)

[13]. WHO. (2009). Dengue. *GUIDELINES FOR DIAGNOSIS, TREATMENT, PREVENTION AND CONTROL.* Retrieved from.

https://www.who.int/publications/i/item/9789241547871

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