

Dengue: Rapid Diagnostic Testing in a Tertiary Care Setting in Butwal, Nepal

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Abstract

Dengue virus (DENV) is spread mostly by biting by the infected female mosquitoes of the species *Aedes aegypti*, that are commonly found in tropical and subtropical zones of globe. Some studies have shown that nearly fifty percent of the globe are susceptible to DENV. Most infections by DENV are asymptomatic. Clinical infections vary from mild fever to serious diseases including hemorrhagic fever and shock syndrome. The main objective of this study was to estimate DENV infection by rapid diagnostic (RDTs) kit methods in a tertiary care setting. This retrospective study was done at Crimson Hospital, Butwal, Nepal from June to November 2019. Serum samples of all dengue fever suspected patients were tested for DENV antigen as Dengue nonstructural protein 1(NS1) and DENV specific antibodies as IgM and IgG by using World Health Organization Good Manufacturing Practices (GMP) certified rapid solid phase immunochromatographic method. All laboratory test results were tabulated by using MS-Excel and analyzed by statistical package for the Social Sciences software version 20. Out of 821 samples tested, 518 were positive, including 322 (62.16%) reactive for dengue NS1 Protein, 121 (23.35%) for IgM, 62 (11.96%) for IgG and 13 (2.50%) were positive for both IgG and IgM antibodies. Our study showed high prevalence of dengue infection during monsoon to post-monsoon and the infection rate was higher in males (71.72%) than in females (53.36%). According to our findings, DENV infection is prevalent in Butwal, Nepal. Early diagnosis, better case management, faster public health response and effective health policy towards the control of mosquito vectors may reduce the dengue burden and must be implemented in Nepal.

Keywords: Dengue Fever, Immunoglobulin G (IgG), Immunoglobulin M (IgM), Non-Structural Protein 1 (NS1), RDTs

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INTRODUCTION

The arthropod-borne virus (arbovirus) *Dengue virus* (DENV) spread mostly biting by infected female mosquitoes of species *Aedes aegypti*. This vector is commonly found in tropical and subtropical zones of the globe. DENV causes significant health impacts mainly in Asia and Latin America. DENV is classified to genus *Flavivirus* and family *Flaviviridae* and presents four serotypes, including DENV-1, DENV-2, DEN-V3 and DENV-4. DENV has three structural proteins, including capsid (C), membrane (M) and envelope (E), and seven nonstructural proteins, including NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5.^{1,2}

Nearly, fifty percent of the globe are susceptible to DENV infection, and it is estimated that 50 million infections occur per year.³ The incubation period of dengue varies from four to ten days and clinical symptoms vary from mild fever to serious disease like dengue hemorrhagic fever (DHC), dengue shock syndrome (DSS).^{4,5}

Nepal is mostly located in the Himalayas, surrounded by China to the north and India to the south, east and west. Nepal's population is estimated at around 26.4 million people, and is unevenly distributed in three ecoregions (terai, hilly and mountain) and 77 districts.⁶ The first cases of dengue in Nepal was first reported from the Terai region in 2004, after the emergence of a dengue outbreak in India.⁷ Nepal has various types of governmental and non-governmental health services. However, neither of them are supplied with molecular diagnostic methods due to high cost.^{8,9} Therefore, rapid diagnostic tests (RDTs) are preferably being used.

METHODS

This retrospective study was conducted from June 2019 to November 2019 at Crimson Hospital, Butwal, Nepal. The laboratory testing was done by using a rapid immune-chromatography kit for NS1, IgM and IgG (Dengue Day 1 Test, manufactured by J. Mitra & Co. Pvt. Ltd. from India).

RESULTS

Serum samples of total 821 patients were

tested for NS1 antigen, and also for IgM and IgG antibodies. Out of the 821 total patients, 492 (60%) were from urban areas and 329 (40%) from rural areas. From 821 patients, 518 (63%) were positive for NS1 or IgM/IgG antibodies (Table 1). Among the 518 dengue-positive patients, 312 (60%) were men and 206 (40%) were women. Higher prevalence of infection by age group were 31-40 years old (n=169, 80.47%), followed by 41-50 years old (n=95, 66.90%) and 21-30 years (n=120, 63.82%) (Table 2).

Among the 518 dengue-positive samples in this study, 322 (62.16%) were positive for NS1 Antigen, 121 (23.35%) for IgM antibodies, 62 (11.96%) for IgG antibodies and 13 (2.50%) were positive for both IgG and IgM antibodies (Table 3). This indicates that the acute infection by DENV (NS1 antigen or positive IgM) was higher when compared to the convalescent infection both IgG and IgM.

Table 1. Epidemiological data on Sero-positivity of DENV infection

Total Samples	Dengue Test Result	Percentage (%)
518	Positive	63.09
303	Negative	36.90

Table 2. Demographic characteristic of DENV infected patients (n=821)

	Number of patients	Positive cases	Percentage (%)
Gender			
Male	435	312	71.72
Female	386	206	53.36
Age Group in years			
01-10	41	16	39.02
11-20	117	61	52.13
21-30	188	120	63.82
31-40	210	169	80.47
41-50	142	95	66.90
51-60	81	39	48.14
61-70	20	8	40.00
71-80	12	5	41.66
81-90	8	4	50.00
91-100	2	1	50.00
Geographical Distribution			
Urban	492	302	61.38
Rural	329	216	65.65

Table 3. Sero-positivity patterns of DENV infection

Diagnostic test	Total Number	Percentage (%)
NS1Ag	322	62.16
IgMAbs	121	23.35
IgGAbs	62	11.96
IgM + IgG	13	2.50
Total	518	100

Table 4. Months wise distribution of DENV infection

Time of sample collection	Number of samples	Positive Cases	Percentage (%)
June	72	28	38.88
July	133	82	61.65
August	207	135	65.21
September	266	189	71.05
October	103	72	69.90
November	40	12	30.00
Total	821	518	100

In this study, prevalence of infection by DENV increased from June (38.88%) to September (71.05%), and decreased in November (30%) (Table 4). Findings reveal that the number of cases was higher during the rainy season (June) when compared to the dry season (December).

DISCUSSION

Dengue fever is a great public health concern in tropical and subtropical regions. After the first reports of dengue fever in 2004 in Nepal, there was a rise and fall of dengue cases with a total 17,992 cases reported between 2018-2019 in 68 districts.^{2,10} According to a nationwide survey conducted from 2016-2019, infection rate was higher in 2018 and 2019. Because of this, Nepal Government implemented prevention and control of dengue all over the country.¹¹ Because of the wide spectrum of unspecific clinical signs and symptoms, clinical diagnosis of dengue is challenging, so laboratory diagnosis is instrumental for a more accurate dengue diagnosis.¹² Virus isolation, RT-PCR and neutralization tests are powerful laboratory tools that provide accurate diagnosis of DENV

infection. However, because these methods require equipped facilities and are often costly, rapid diagnostic tests based on certified rapid solid phase immunochromatographic methods are alternatives that can also provide valuable information regarding DENV infection. Detection of IgM or IgG antibodies for DENV infection that indicates previous exposure to DENV is a commonly used method. The detection of specific IgM antibodies suggests recent infection and often can be done using a single drop of serum. From three days to two month of infection, IgM antibodies can be detected in blood. Likewise, IgG antibodies can be detected after from one week of infection to lifetime.^{1,9,13,14,15}

Our findings demonstrated that 63.09% of the patients were serologically positive which is similar to another study where 131/164 (79.9%) patients were IgM and/or IgG positive.¹⁶ Another study from Nepal showed that 57% of patients were positive for DENV infection.¹⁷ In Thailand, 65% of patients were positive by dengue rapid test.¹⁸ In our study, of the 518 dengue-positive cases, 312 (60%) were male and 206 (40%) were female. These results indicate that 71.71% of males tested were positive and 53.36% of females tested were positive for DENV infection. These results were similar to other studies.^{19,20,21}

In our investigation, the most affected age group was 31-40 years old (n=169, 80.47%) followed by 21-30 years old (n=120, 63.82%). Another similar study showed a higher positivity rate (16.4%) in the 20-40 year-old group.²²

While observing the period of infection, the number of positive cases gradually increased from June to November. The seasonality of dengue transmission with increased transmission during monsoon activity was consistent with reports of dengue transmission elsewhere. The presence of Stagnant water during rainy season promotes the reproduction of mosquito vectors and consequently higher number of dengue cases.²³⁻²⁵ Early implementation of all safety measures to control the spread of dengue should be strictly referred during monsoon and post monsoon months.

CONCLUSION

Life threatening dengue is mostly

prevalent in tropical and subtropical areas of the globe. In Nepal, dengue is frequently widespread from the terai regions. Fatality rate can be higher than 40% in cases of dengue hemorrhagic fever and dengue shock syndrome. The present study shows 63.09% of prevalence in a tertiary care setting of Butwal, Nepal in 2019. Higher prevalence in men than in women merits further investigation. The early identification of dengue viral diseases by using a rapid diagnostic (RDTs) kit, certified by WHO is essential for all developing countries, including Nepal. Furthermore, Nepal government should establish effective health policy and vector control strategies, including steps for awareness of prevention, better case management and faster public health response to reduce the disease burden.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

RBK conceived the research idea and designed the study. BN, JP, GPC and KK performed data analysis. APP and RG performed data interpretation. RBK, DP, SKG, APP and RG wrote the manuscript. All authors read and approved the final manuscript for publication.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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