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Serotype of Dengue Virus Causing Dengue Outbreak in Kathmandu

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ABSTRACT

Background: The most dengue cases ever recorded in Nepal were reported in 2019, the greatest number to date. The incidence of dengue infections in Kathmandu is examined from an epidemiological standpoint in this paper. Since its initial introduction in 2004, dengue cases—including some significant outbreaks—have been consistently reported in Nepal. The purpose of this study was to identify and characterise the dengue virus (DENV) at the molecular level in dengue patients.

Methods: This study had 579 patients in all. Dengue patients who visited the National Public Health Laboratory provided demographic, clinical, and laboratory data. RT-PCR analysis was performed after immunochromatographic detection assays to confirm dengue infection.

Results: By immunochromatographic screening, 234 (40.41%) of the 579 patients tested positive for the Dengue virus. Of them, 185 (79.05%) samples tested positive for the NS1 antigen, 36 (15.38%) tested positive for IgM, and 1 (0.42%) tested positive for IgG. Remarkably, 345 samples tested negative for dengue virus, while 12 (5.12%) tested positive for both NS1 and IgM. 93 of the 185 NS1-positive samples underwent real-time PCR characterisation. We discovered that the most common serotype causing the 2019 outbreak was DENV-2, 90 (96.77%). Interestingly, co-infection with DENV 1 and DENV 3 was reported in one patient, while two samples tested negative for the Dengue virus.

Conclusions: According to our research, the main serotype responsible for the significant epidemic in Nepal in 2019 was DENV 2. Programs for disease control will benefit from this knowledge as it helps them comprehend molecular characterisation and its evolving trend.

Keywords: Dengue; DENV 2; Kathmandu; RT-PCR; serotype.

INTRODUCTION

Aedes aegypti or Aedes albopictus bites cause dengue, a serious public health issue in tropical and subtropical areas that affects about 390 million people yearly, of whom 96 million have clinical symptoms.[1,2] From asymptomatic episodes to severe dengue hemorrhagic fever (DHF), the disease can present itself in a variety of ways. The symptoms of dengue fever (DF), which is a self-limiting infection, include fever, rash, joint pain, weakness, and thrombocytopenia.[2] The causal agent is the dengue virus, which is composed of four antigenically different serotypes (DENV 1-5).[3] Climate change, fast urbanization, poor wastewater management, and inadequate mosquito control all contribute to the disease's global spread. Major outbreaks, observed in several developing nations, including Cuba (1977-79, 1997), New Delhi (1996), Taiwan (2002), and Brazil (2008), have negatively impacted economic and social well-being.[4]

Climate variables such as rainfall, temperature, humidity, and vector abundance all influence the virus's potential to propagate.[5] The virus's microevolution, which results in the formation of more aggressive genotypes, as well as shifts in serotypes, genotypes, and lineages, has been linked to disease severity variations[6].This emphasizes the importance of rigorous virological surveillance to detect the emergence of harmful DENV strains. Despite dengue's global effect, there is little knowledge on the genetic epidemiology of DENV strains in specific areas, such as Nepal. Comprehensive studies are critical for producing effective vaccinations and identifying potential genotypic alterations. Understanding the

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molecular epidemiology of circulating DENV strains is critical for preparedness against future outbreaks.

METHODS

This is a cross-sectional and sequential study to determine dengue serotype using real-time RT-PCR in NS1 antigen positive dengue cases. This investigation was conducted at the vaccine preventable illness unit of the National Public Health Laboratory, Teku, Kathmandu, using samples taken in October 2019 (peak Dengue cases). Ethical approval was acquired from Nepal Health Research Council (Reference no 1501). Serum samples, demographics, and clinical history were gathered from feverish patients at the National Public Health Laboratory for Dengue testing. Dengue was tested by immunochromatography in serum using a fast kit that detects Ns1 antigen. IgM and IgG NS1 positive samples were retained for molecular characterization of the virus.

The RNA was extracted manually using the QAIamp® Viral RNA micro kit (QIAGEN GmbH, Hilden, Germany), according to the manufacturer's instructions. The CDC Real-time RT-PCR protocol for Dengue virus detection and characterization includes a panel of oligonucleotide primers and dual-labeled hydrolysis (taqman®) probes for use in real-time RT-PCR assays for the qualitative detection and characterization of Dengue virus in serum samples. The Dengue virus primer and probe set were designed for universal detection of (DENV 1-DENV 4). The DENV 1, DENV 2, DENV 3, and DENV 4 RT-PCR experiments were performed in 25µL reaction mixes using 5µL template RNA. Primers and probes were acquired from the CDC. Amplification and detection were carried out using a Step One Plus real-time PCR system (Bio-Rad CFX 96). Thermocycling settings were as follows: reverse transcription at 50°C for 30 minutes, inactivation at 95°C for 2 minutes, 45 cycles of fluorescence detection at 95°C for 15 seconds, and annealing at 60°C for 1 minute. The baseline and threshold were established using Step One Software's auto-baseline and threshold feature (Bio-Rad CFX 96). Samples were judged positive if the target amplification was detected within 40 cycles. Data were collected only after each specimen produced valid results. The data were entered into both Microsoft Excel 2013 and IBM SPSS Statistics Version 20.

RESULTS

Data were collected only after each specimen produced valid results. The data were entered into both Microsoft Excel 2013 and IBM SPSS Statistics Version 20. (Table 1& Fig 1).

Table1. Result of Dengue Virus by Rapid Immunochromatographic.

Total samples	NS1 Positive	IgM Positive	IgG Positive	NS1 +IgM Positive	Negative
579	185	36	1	12	345

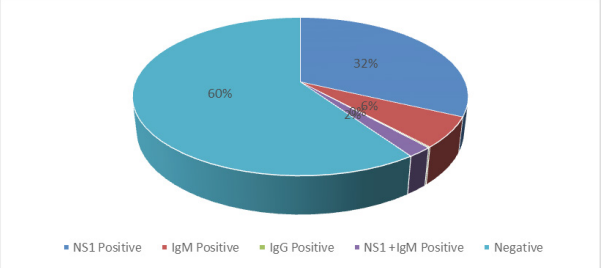

Figure1.Pie chart showing result of Dengue Virus.

93 NS1 positive samples were chosen for Real-Time PCR assays (n=93). (Table 2& Fig 2).

Table 2. Result of Dengue Virus by Real Time PCR.

Total samples	Dengue Type 2 Positive	Dengue Type 2 & 3 Positive	Dengue Negative
93	90	1	2

Out of 93 samples, 90 samples revealed Dengue Type 2, two samples were negative, and one sample showed coinfection with both type 2 and type 3 in Table 2.

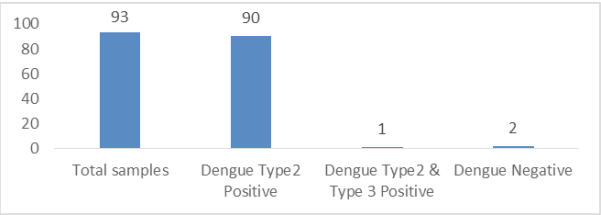

Figure 2. Result of Dengue Virus by Real Time PCR.

DISCUSSION

The prevalence of dengue infections in Nepal has increased over time, with cases of all four dengue virus serotypes confirmed at various times.[7] In our analysis, the predominant strain that caused the dengue outbreak in Kathmandu in 2019 was found to be Dengue type 2. From July to September 2019, Nepal's Epidemiology and

Disease Control Division (EDCD) recorded 7370 dengue positive cases, six of which were fatal[8] . The dengue virus positive rate in 2019 was 38.17%[8] . In our investigation, the positivity rate of Dengue virus was 40.41%, which was higher than the previously published study.

Traditional dwellings, open water bodies, and poor sewage treatment have all contributed to the breeding grounds of mosquitos.[9] Furthermore, high population density and uncontrolled urbanization may have helped the illness spread quickly. The growth of the vector is closely proportional to environmental elements such as monsoons, temperature changes, and humidity. Dengue cases predominated in Nepal between July and December.[10] A 2015-2016 meteorological study indicated that Kathmandu's monsoon season temperatures ranged from 19.6 ± 0.62 °C (minimum), with rainfall totaling 370.7 ± 157.20 mm and relative humidity at 82.3 ± 1.50%, all of which had an impact on the vector indices.[10] A study found that adults have a higher rate of clinical dengue than young children.[11] In our study, there were more female patients than male patients. With over 14 000 instances reported from 67 districts, 2019 was a record year. It's worth noting that some of these districts had never faced dengue before. The population's increased travel and a number of mosquito-related factors may be contributing to the epidemic's expansion and explaining the spread of DENV to new places.[12] During the 2019 dengue outbreak, more than 2500 cases were reported in Kathmandu, the country's capital, setting a new record. This demonstrates that dengue is a disease with a significant local presence in the Kathmandu valley. There are numerous Aedes mosquitos in Nepal, particularly in Kathmandu (1400 meters above sea level). Kathmandu is growing into a suitable breeding ground for Aedes mosquitoes, possibly due to the city's rapid and unplanned urbanization, population increase, individuals moving from dengue-endemic regions, and climate change.[13] During severe dengue outbreaks in 2010, 2013, 2016, 2017, and 2018, DENV 1 (2010 and 2016) and DENV 2 (2013) were discovered. Given that the same Aedes mosquitos disseminate viruses like CHIKV, ZIKV, and yellow fever in dengue-endemic areas, it is high time for Nepal's disease control officials to explore additional mosquito-borne viral diseases among febrile patients.[14]

The present investigation was conducted using cross-sectional data from patients' laboratory records. Clinical data was not collected. A study was conducted on patients from Kathmandu valley visiting the National Public Health Laboratory, Teku.

CONCLUSIONS

Our data indicate that DENV 2 was the primary serotype responsible for the 2019 large outbreak in Nepal. In Nepal, climate change, unplanned development, and deteriorating environmental circumstances have resulted in an increase in the number of cases in recent years. Continued epidemiological and entomological surveillance, as well as the control of factors that contribute to outbreaks, will serve to lessen the disease's public health burden.

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