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Determination of the Proportion of Dengue Serotypes by RT-PCR and Correlation of Its Severity as per Recent WHO Classification at a Tertiary Care Hospital, Bhavnagar

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Abstract

Background: Dengue fever, caused by the dengue virus (DENV), is a major global health concern, particularly in tropical and subtropical regions. India, being hyperendemic, has witnessed an increasing burden of dengue, with outbreaks peaking post-monsoon. This study aimed to identify the prevalent dengue serotype and assess its correlation with disease severity based on the WHO 2009 classification.

Aim: To identify the prevalent dengue serotype and to correlate its severity of serotype clinically according to recent WHO classification.

Materials and Methods: This prospective study was conducted at the Viral Diagnostic and Research Laboratory, Department of Microbiology, Government Medical College, Bhavnagar, from September 2022 to August 2023. A total of 148 patients with suspected dengue fever, confirmed by NS1 antigen and/or IgM ELISA, were included. Dengue serotyping was performed using RT-PCR. Clinical severity was categorized into dengue without warning signs, dengue with warning signs, and severe dengue per WHO guidelines.

Results: Out of 148 suspected cases, 87 (58%) were RT-PCR-confirmed for dengue. The most affected age group was 16-30 years (49%), with peak incidence in October. DENV-2 was the predominant serotype (57 cases, 65%), followed by DENV-4 (18 cases, 20%), DENV-1 (4 cases, 5%), co-infection of DENV-2 and DENV-4 (7 cases, 9%), and a rare triple infection (DENV-1, DENV-2, and DENV-4) in 1 case (1%). Clinical severity analysis revealed that DENV-2 was associated with more severe cases (12 with warning signs, 9 with severe dengue), followed by DENV-4 (3 with warning signs, 1 severe case).

Conclusion: DENV-2 was the predominant serotype and strongly associated with severe cases, reinforcing its clinical significance. Continuous serotype surveillance, vector control, and RT-PCR-based diagnostic integration into routine dengue surveillance are critical for disease management and vaccine strategies.

Keywords: Dengue Virus, Serotyping, RT-PCR, DENV-2, Severe Dengue, Epidemiology

Introduction

Dengue virus is the most prevalent arbovirus in the world, more than 100 million people infected annually[1]. The incidence of dengue has grown dramatically around the world in recent decades, with cases reported to WHO increasing from 505, 430 cases in 2000 to 5.2 million in 2019. The highest number of dengue cases was recorded in 2023, affecting over 80 countries in all regions of WHO. Since the beginning of 2023 ongoing transmission, combined with an unexpected spike in dengue cases, resulted in a historic high of over 6.5 million cases and more than 7300 dengue-related deaths reported [2]. This disease has become established (endemic) in nearly 100 countries across Africa, the Americas, Eastern Mediterranean, Southeast Asia, and the Western Pacific [3].

Dengue infection can cause a wide variety of symptoms, ranging from no noticeable illness (asymptomatic) to severe cases with complications. These complications can involve increased leakage from blood vessels (vascular permeability), problems with blood clotting (homeostasis disorders), and damage to organs (organ impairment) [4].

The dengue virus is composed of about 11,000 nucleotides. This genetic material is single-stranded RNA and wrapped in a protective envelope. They consist of untranslated regions (UTRs) at both ends, like margins in a book [5]. The code is divided into two main sections, separated by a non-coding region. These sections are called open reading frames (ORFs), and they contain the blueprints for building the virus. The first ORF codes for the virus's structural proteins, the building blocks that form its outer shell and core. These proteins are called capsid (C), envelope (E), and membrane (M) proteins 5. The second ORF codes for non-structural proteins, which are like molecular machines that the virus uses to take over the host cell and replicate itself. There are seven of these non-structural proteins, designated NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5 5.

Diagnosing dengue involves both clinical evaluation and laboratory testing to confirm the presence of the virus. Clinically, patients typically present with high fever, severe headache, retro- orbital pain, joint and muscle pain, rash, and mild bleeding [1]. These symptoms, along with a history of travel to endemic areas, can raise suspicion for dengue. Laboratory tests are crucial for confirmation and include serological tests such as IgM ELISA to detect dengue-specific antibodies, and rapid diagnostic tests that can quickly identify the presence of the virus. Molecular tests like reverse transcription-polymerase chain reaction (RT-PCR) detect viral RNA, providing a definitive diagnosis especially useful in the early stages of infection [1,2]. Antigen detection tests, such as the NS1 antigen test, are also used within the first week of symptom onset. Complete blood count (CBC) tests reveal characteristic changes in dengue, such as a drop in platelet count and an increase in haematocrit levels, indicating plasma leakage. Accurate and timely diagnosis is essential for effective patient management and controlling the spread of dengue [3].

Aim & Objectives

Aim

To identify the prevalent dengue serotype and to correlate its severity of serotype clinically according to recent WHO classification.

Objectives

- Determination of different serotypes by RT- PCR
- Correlation of serotype-specific dengue virus infection with clinical manifestations

Materials and Methods

Study Design and Setting

A prospective study was conducted at the Viral Diagnostic and Research Laboratory, Department of Microbiology, Government Medical College, Bhavnagar, from September 2022 to August 2023.

Sample Collection and Inclusion Criteria

A total of 148 patients presenting with symptoms of dengue fever were included. Cases were confirmed using NS1 antigen and/or IgM ELISA.

Laboratory Diagnosis

1. Serological Testing

NS1 antigen and IgM ELISA were used for preliminary screening.

2. RT-PCR for Serotyping

Viral RNA was extracted using a commercial extraction kit.

A multiplex RT-PCR assay was conducted to differentiate DENV-1, DENV-2, DENV-3, and DENV-4.

3. Clinical Classification

Patients were categorized per WHO 2009 criteria into:

Dengue without warning signs

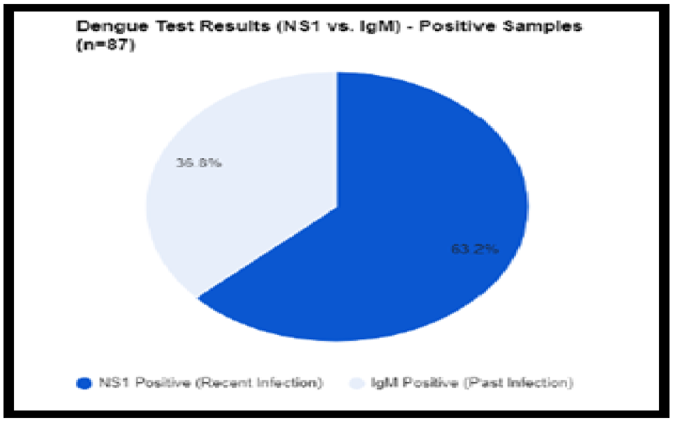
Dengue with warning signs

Severe dengue

Results

Dengue Positivity Rate

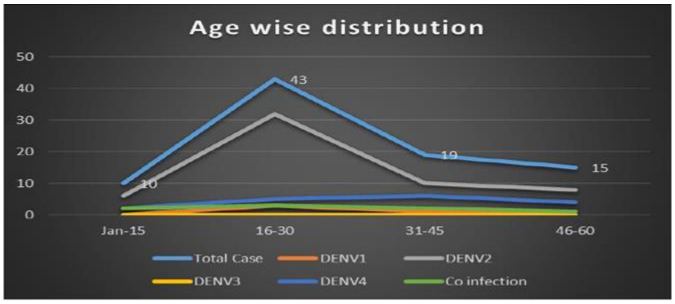
Among 148 suspected cases, 87 (58%) were confirmed as dengue-positive via RT-PCR.



Age Distribution

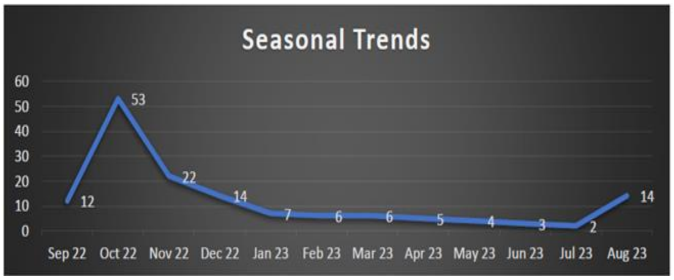
The most affected age group was 16-30 years (49%).

Age Group	Total Cases	DENV1	DENV2	DENV3	DENV4	Co infection
1-15	10	0	6	0	2	2
16-30	43	3	32	0	5	3
31-45	19	1	10	0	6	2
46-60	15	0	8	0	4	1



Seasonal Distribution

The highest incidence was observed in October, indicating a post-monsoon surge.



Dengue Serotype Distribution

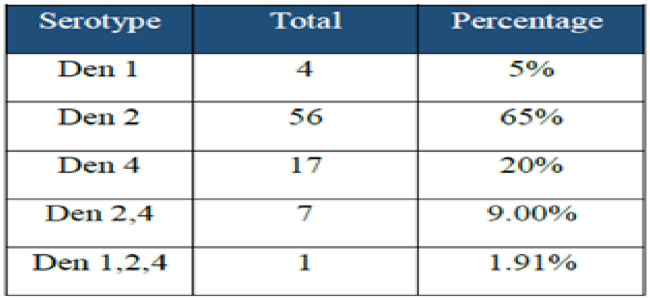
DENV-2: 57 cases (65%)

DENV-4: 18 cases (20%)

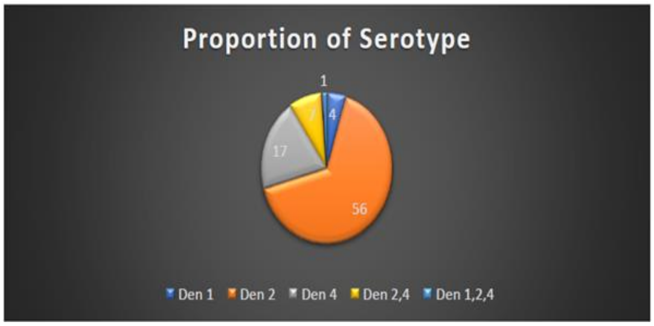
DENV-1: 4 cases (5%)

Co-infection (DENV-2 & DENV-4): 7 cases (9%)

Triple infection (DENV-1, DENV-2, and DENV-4): 1 case (1%)



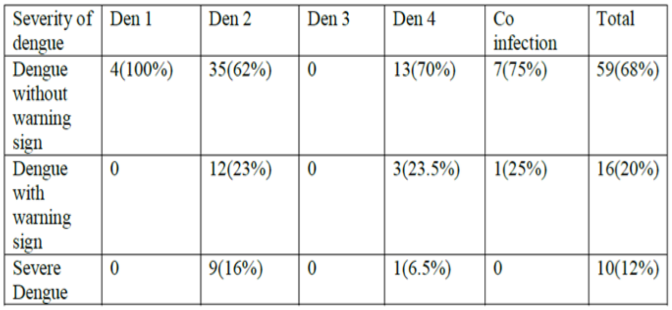
Serotype	Total	Percentage
Den 1	4	5%
Den 2	56	65%
Den 4	17	20%
Den 2,4	7	9.00%
Den 1,2,4	1	1.91%



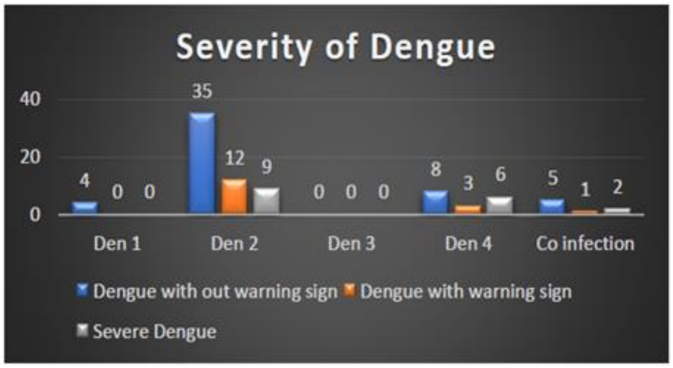
Clinical Severity and Serotype Correlation

DENV-2: 12 cases had warning signs, and 9 developed severe dengue.

DENV-4: 3 cases had warning signs, and 1 developed severe dengue.



Severity of dengue	Den 1	Den 2	Den 3	Den 4	Co infection	Total
Dengue without warning sign	4(100%)	35(62%)	0	13(70%)	7(75%)	59(68%)
Dengue with warning sign	0	12(23%)	0	3(23.5%)	1(25%)	16(20%)
Severe Dengue	0	9(16%)	0	1(6.5%)	0	10(12%)



Discussion

In this study in majority of the positive samples were from NS1 positive patients. There is similar study Aviral Gupta et al 6 conveying the same data. There are multiple other studies happened in various part of the country sharing the same [7,8,9]. The dengue disease has 3 stages Febrile stage, Critical stage, Recovery stage. Active viral replication will happen during the febrile phase and followed by defervescence, where the cessation of viremia will happen. This stage is usually will occur after 7,8 days of illness. And usually the NS 1 samples are tested with in 5 t days of onset of illness This could be the reason for more positivity of detecting RT PCR in NS 1 positive patient.

In our study males are affected more than females. The result of this study was found in accordance with other studies which is conducted in Gujrat Tanmay et al [10] suggest that the males are more infected than female. Similar observations were also reported by recent studies. Other studies conducted in different regions of Asia, Goh et al [11], Rosa T et al [12] also reported the similar observations In a global study which is conducted in southeast Asia also suggesting the same [14].

In the present study, patients in the age group of 16-30 years were more affected by Dengue fever followed by the patients in the age group of 31-45 years, study conducted by Suneel Kumar Et al [15] sharing the same data. These findings are supported by a report published by Tanmay et al [10], Pooja et al [15], Bindu Jadeja et al [7] which stated that the patients between age group of 15-34 years were most affected by Dengue fever. Various studies from india also demonstrated maximum number of infections in the same age group as found in the current study [16,17,18].

Usually, dengue epidemics are reported to occur during the warm, humid, and rainy seasons, because it Favors mosquito growth and shorten the extrinsic incubation period [14,15]. According to the intensity of rainfall, weather data was divided in three periods namely; pre monsoon period: from February- May, monsoon period: from June –September and post monsoon period: from October – January. In current study, the largest proportion of serologically positive cases was recorded during the post monsoon period. In this study, dengue cases begin to start from the month of August, and maximum number of cases was reported in the month of October. Increased mosquito breeding, Optimal temperature and humidity can explain the same finding. Here, we can draw a conclusion that dengue infections frequently occurred during the monsoon and post monsoon periods. Although in our study there is a surge in case at post monsoon, comparable with study conducted by Srinivas Rao et al [19], Poornima et al [20] ,and study from New Delhi and Bangladesh regarding the seasonal trends also stating the same [21,22].

In this study out of 87 positive patients 56 is DEN 2 serotype which is 64% of the total positive, so we can understand that Den 2 serotype is highest in proportion, same highest prevalence of DEN 2 serotype observed in a study conducted by ICMR VRDL, even in that study says that prevalence DEN 2 serotype is more in Gujrat [23]. There are various other studies Barde, P.V [24] et al, Gaurav Badoni et al [25] Srisena et al [26] concluding as the prevalent serotype is DEN 2. Considering the other studies which had conducted globally by Creusa Rachel et al [27],Nizam Khalid et al [28], Jih jen Tsal et al [29] also giving to the same result. The predominance of dengue serotype 2 (DENV-2) in certain outbreaks can be attributed to several factors such as Viral Fitness and Evolution i.e. DENV-2 may possess genetic variations that enhance its fitness, allowing it to replicate more efficiently in mosquito vectors and human hosts. These variations can make DENV-2 more competitive compared to other serotypes.

In our study DENV 4 is the second common serotype ,Suneel kumar et al [30] sharing the same ,and study conducted in Tamilnadu [31] and eastern part [32] showing the emergence of dengue 4 apart from the reason discussed shift in the serotype pattern and probable mutation in the virus and chances of cross reaction could attribute to the same.

DEN 2 is the most common serotype which is presented with hemorrhagic manifestations, even though the actual cause is unknown, there are numerous studies which is concluding the same, Pooja et. al [15] , Tanmay et. Al [10], Aviral Gupta et. al, Gaurav Badoni et al, .There are international studies suggesting the high severity of dengue serotype like Study from Brazil, Singapore, Malasia [27,28],. The cosmopolitan distribution of DENV 2 is evident from the above studies. Virulence, Strong immune response could be the reason for the disease severity. Immunopathogenesis: DENV-2 has been found to induce a stronger immune response in some individuals, which can lead to an exaggerated immune reaction known as cytokine storm. This excessive immune response can contribute to increased vascular permeability, leading to plasma leakage and potentially causing hemorrhagic manifestations. Different dengue virus serotypes can vary in their ability to infect and replicate within host cells, as well as in their capacity to evade the immune response.

Coming to the other serotype DEN 4 is the most common second serotype in our study, even there are other studies which is stating the same, [12,26,29] and considering the clinical manifestation it is the second common serotype causing haemorrhagic manifestation. A study which is conducted in a tertiary care centre Gujrat also stating the same.

There are other studies showing that even the patient is infected with multiple serotype at the same time can have severe symptoms other than constitutional manifestaions. Sirisena et. al.[26] Anoop et.al.[33] Mammon et.al [34] sharing the same data with us. The justification for this findings lies in the complex interplay of the immune response, the specific characteristics of the viruses involved, the population studied, and the methodological approaches. Understanding these factors allows for a more comprehensive interpretation of the seemingly contradictory results. Further research is needed to elucidate the precise mechanisms and conditions under which multiple serotype infections lead to milder or more severe diseases.

Recommendations

1. Strengthening vector control measures, particularly before and after monsoon seasons.
2. Incorporating RT-PCR-based serotyping into routine surveillance.
3. Conducting genetic studies on evolving serotype dominance and its implications for vaccine efficacy.

Conclusion

In conclusion, dengue fever remains a significant global health challenge, affecting millions annually across multiple regions. This study contributes valuable insights into the epidemiology and clinical presentation of dengue, highlighting the prevalence of DEN 2 serotype and its association with clinical symptoms such as fever and body pain.

Molecular analysis and serotyping of dengue virus were found to yield more reliable, accurate, and sensitive results compared to serological testing. Coinfections with multiple serotypes were not linked to increased disease severity in this study.

The expansion of dengue is expected to rise due to factors such as the modern dynamics of climate change, travel, globalization, socioeconomics, trade, settlement and also because of continuous viral evolution. Dengue virus shows a very high mutation rate, due to existence of multiple serotypes, genotypes, lineages and accumulating genomic variation, hence it is important to track the genomic changes in the virus.

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