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VIRAL LOAD PATTERNS IN DENGUE INFECTION: DIAGNOSTIC AND CLINICAL SIGNIFICANCE OF REAL-TIME RT-PCR—A COMPREHENSIVE REVIEW

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ABSTRACT

Dengue fever is one of the fastest-growing mosquito-borne viral diseases worldwide and poses a significant public health challenge, particularly in tropical and subtropical countries. The disease is caused by four antigenically distinct dengue virus (DENV) serotypes (DENV-1 to DENV-4), which are transmitted primarily by Aedes aegypti mosquitoes. Clinical manifestations range from asymptomatic infection and uncomplicated dengue fever to severe dengue, including dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Viral load has emerged as an important biomarker for understanding disease progression, predicting severity, and evaluating patient prognosis. Real-time reverse transcription polymerase chain reaction (RT-PCR) has become the gold standard for early dengue diagnosis because of its high sensitivity, specificity, and ability to quantify viral RNA during the acute phase of infection. This review summarizes current evidence regarding dengue epidemiology, viral pathogenesis, viral load kinetics, diagnostic approaches, and the clinical significance of viral load estimation using RT-PCR. Furthermore, the review discusses recent advances in molecular diagnostics, laboratory biomarkers, and public health strategies for dengue prevention and control. Understanding viral load dynamics provides valuable insights into disease progression and facilitates timely clinical intervention, thereby reducing morbidity and mortality associated with severe dengue.

KEYWORDS: Dengue virus, Viral load, RT-PCR, Molecular diagnosis, Dengue fever, NS1 antigen, Real-time PCR, DENV.

1. INTRODUCTION

Dengue is an acute mosquito-borne viral disease caused by the dengue virus (DENV), a member of the genus Flavivirus within the family Flaviviridae. It represents one of the most rapidly expanding vector-borne diseases globally, affecting millions of individuals each year. According to the World Health Organization (WHO), approximately half of the world's population is currently at risk of dengue infection, with nearly 100–400 million infections estimated annually. The disease is endemic in more than 100 countries, particularly across Asia, Latin America, and Africa, where favorable climatic conditions facilitate the proliferation of Aedes mosquito vectors.

India contributes substantially to the global dengue burden due to rapid urbanization, population growth, climate change, inadequate vector control, and increased human mobility. Seasonal outbreaks occur frequently during and after the monsoon season, placing enormous pressure on healthcare systems. Although the majority of infections are self-limiting, a proportion of patients develop severe complications such as dengue hemorrhagic fever (DHF), dengue shock syndrome (DSS), severe plasma leakage, bleeding manifestations, and multiple organ dysfunction.

Early diagnosis remains the cornerstone of effective dengue management. Traditional diagnostic methods based on serological detection of IgM and IgG antibodies are limited during the initial days of infection because antibody production begins only after several days of illness. Consequently, molecular diagnostic techniques, particularly real-time reverse transcription polymerase chain reaction (RT-PCR), have become increasingly important because they enable direct detection of viral RNA during the early viremic phase. Viral load estimation has further enhanced understanding of dengue pathogenesis by demonstrating associations between high viral replication and increased disease severity.

This review critically examines current literature on dengue viral load kinetics, molecular diagnostic approaches, and the clinical importance of RT-PCR in disease management. It also highlights recent developments in laboratory diagnosis and discusses future directions for improving dengue surveillance and patient outcomes.

2. Global Epidemiology of Dengue

Dengue has evolved into one of the world's most important arthropod-borne viral infections. Over the past five decades, its incidence has increased dramatically owing to globalization, rapid urban expansion, increased international travel, inadequate sanitation, and climate variability. The World Health Organization estimates that approximately 3.9 billion people

living in more than 120 countries remain at risk of dengue infection.

South-East Asia bears a disproportionate share of the global disease burden. Countries including India, Bangladesh, Sri Lanka, Thailand, Indonesia, Malaysia, Vietnam, and the Philippines report recurrent epidemics involving multiple circulating serotypes. Hyperendemic transmission of all four dengue serotypes has become increasingly common, resulting in repeated infections and a greater risk of severe dengue due to antibody-dependent enhancement.

In India, dengue cases have increased considerably over the past two decades. Urban centers experience annual outbreaks, while rural regions have also witnessed expanding transmission owing to changing ecological conditions and inadequate vector control measures. Continuous circulation of different serotypes contributes to recurrent epidemics and complicates disease surveillance.

The emergence of multiple serotypes within the same geographical area presents significant clinical challenges because secondary infection with a heterologous serotype substantially increases the likelihood of severe disease. Consequently, molecular surveillance of circulating serotypes has become an important component of national dengue control programs.

3. Dengue Virus Structure and Transmission

Dengue virus is a positive-sense, single-stranded RNA virus approximately 11 kb in length. Its genome encodes three structural proteins—capsid (C), membrane (M), and envelope (E)—along with seven non-structural proteins (NS1–NS5). The envelope glycoprotein plays a critical role in viral attachment, host-cell entry, and induction of neutralizing antibodies.

Transmission occurs primarily through the bite of infected female Aedes aegypti mosquitoes, although Aedes albopictus also contributes to disease spread in several regions. Following ingestion of viremic blood, the virus undergoes replication within the mosquito before migrating to the salivary glands. During subsequent feeding, infectious viral particles are transmitted into human skin, initiating infection. The intrinsic incubation period in humans ranges from approximately 3 to 14 days before symptom onset. Rare modes of transmission include blood transfusion, organ transplantation, vertical transmission from mother to fetus, and needlestick injuries.

Following inoculation, dengue virus infects dendritic cells, macrophages, monocytes, hepatocytes, endothelial cells, and other susceptible cells. Viral replication within these cells results in systemic dissemination through the lymphatic and circulatory systems, contributing to the wide spectrum of clinical manifestations observed in infected individuals.

4. Pathogenesis and Viral Load Dynamics

The pathogenesis of dengue virus (DENV) infection is a complex interplay between viral replication, host immune responses, and inflammatory mediators that ultimately determines disease severity. Following the bite of an infected Aedes mosquito, the virus is inoculated into the dermis, where it initially infects Langerhans cells, dendritic cells, macrophages, and monocytes. These infected cells migrate to regional lymph nodes, allowing viral dissemination into the bloodstream and lymphatic circulation. Subsequently, DENV infects various target cells, including hepatocytes, endothelial cells, splenic macrophages, and bone marrow cells, resulting in systemic infection and widespread immune activation.

One of the characteristic features of dengue infection is viremia, during which viral replication reaches its peak within the first few days after symptom onset. Viral RNA can generally be detected in serum for approximately four to five days following the onset of fever, making this period optimal for molecular diagnosis using real-time RT-PCR.

Disease severity depends not only on viral replication but also on the host immune response. During secondary infection with a different DENV serotype, pre-existing non-neutralizing antibodies facilitate viral entry into Fc receptor-bearing immune cells through **antibody-dependent enhancement (ADE)**. This process increases intracellular viral replication, resulting in higher viral loads, exaggerated cytokine release, endothelial dysfunction, and increased vascular permeability. These mechanisms contribute significantly to the development of dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS).

The dengue non-structural protein 1 (NS1) also plays a major role in disease pathogenesis. Circulating NS1 protein directly damages endothelial cells and activates inflammatory pathways, leading to plasma leakage, complement activation, coagulation abnormalities, and tissue injury. Patients with severe dengue frequently exhibit elevated concentrations of NS1 and higher viral loads than those with uncomplicated dengue fever. Increased production of inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), interleukin-10 (IL-10), and interferon-gamma (IFN-γ) further amplifies vascular leakage and systemic inflammation.

High viral load has consistently been associated with severe clinical outcomes. Several clinical studies have demonstrated that patients presenting with higher plasma viral RNA concentrations during the early febrile phase are more likely to develop thrombocytopenia, elevated hematocrit, liver dysfunction, plasma leakage, and shock. Viral load therefore serves as an important prognostic biomarker, allowing clinicians to identify high-risk patients before the onset of life-threatening complications.

5. Clinical Manifestations and Laboratory Findings

The clinical spectrum of dengue infection ranges from asymptomatic disease to severe multisystem involvement. After an incubation period of approximately 4–7 days, patients typically develop sudden onset of high-grade fever accompanied by severe headache, retro-orbital pain, myalgia, arthralgia, nausea, vomiting, and maculopapular rash. These manifestations characterize the **febrile phase**, which generally lasts for two to seven days.

Most patients recover without complications; however, a proportion progress to the **critical phase**, usually occurring between days four and seven of illness. This stage is characterized by increased vascular permeability resulting in plasma leakage, pleural effusion, ascites, hypotension, and shock. Warning signs include persistent vomiting, severe abdominal pain, mucosal bleeding, hepatomegaly, lethargy, restlessness, rapid decline in platelet count, and rising hematocrit. Without prompt recognition and appropriate fluid management, these patients may rapidly develop DSS or DHF, both of which are associated with significant mortality.

Laboratory abnormalities evolve throughout the disease course. Leukopenia and thrombocytopenia are among the earliest hematological findings, while progressive hemoconcentration reflects plasma leakage. Elevated liver enzymes, particularly alanine aminotransferase (ALT) and aspartate aminotransferase (AST), indicate hepatic involvement. Coagulation abnormalities, including prolonged activated partial thromboplastin time (APTT) and reduced fibrinogen levels, may occur in severe disease and contribute to bleeding manifestations.

Several biomarkers have been investigated for predicting disease severity. High viral RNA concentration, elevated NS1 antigen levels, increased hematocrit, persistent thrombocytopenia, elevated liver enzymes, complement activation products, and inflammatory cytokines have all demonstrated potential prognostic value. However, among these markers, viral load measured by real-time RT-PCR remains one of the earliest and most reliable indicators of disease progression during the acute phase.

6. Molecular Diagnosis and Clinical Importance of RT-PCR

Accurate diagnosis during the early phase of dengue infection is essential because clinical manifestations overlap considerably with other febrile illnesses, including malaria, chikungunya, leptospirosis, influenza, and COVID-19. Laboratory confirmation therefore plays a central role in patient management and outbreak surveillance.

Serological methods, including detection of NS1 antigen, IgM antibodies, and IgG antibodies

using enzyme-linked immunosorbent assay (ELISA), remain widely used because they are relatively inexpensive and suitable for routine laboratory practice. Nevertheless, antibody-based assays possess limited diagnostic utility during the earliest days of infection when antibodies have not yet developed.

Real-time reverse transcription polymerase chain reaction (RT-PCR) has emerged as the preferred molecular diagnostic method for early dengue detection. This technique involves reverse transcription of viral RNA into complementary DNA (cDNA), followed by exponential amplification using dengue-specific primers and fluorescent probes. Because viral RNA is detectable before antibody production, RT-PCR provides excellent sensitivity during the first five days of illness.

Modern multiplex real-time RT-PCR assays permit simultaneous detection and differentiation of all four dengue virus serotypes within a single reaction. This capability is particularly valuable during outbreaks because circulating serotypes influence disease epidemiology, clinical severity, and public health responses. The differentiation of DENV-1, DENV-2, DENV-3, and DENV-4 assists epidemiological surveillance and facilitates monitoring of serotype shifts over time.

Beyond diagnosis, quantitative RT-PCR provides viral load measurements that correlate closely with disease severity. Patients exhibiting lower cycle threshold (Ct) values generally possess higher viral RNA concentrations and are more likely to develop severe clinical manifestations. Consequently, viral load estimation has become an important prognostic tool, enabling clinicians to identify patients requiring closer monitoring, aggressive fluid management, and hospitalization.

Furthermore, RT-PCR contributes significantly to research investigating antiviral therapies, vaccine development, viral evolution, and transmission dynamics. Continued advances in molecular diagnostics, including automated nucleic acid extraction, digital PCR, and point-of-care molecular testing, are expected to improve diagnostic accessibility and enhance dengue surveillance worldwide.

7. Treatment and Clinical Management

Currently, there is no universally effective antiviral therapy for dengue virus (DENV) infection, and patient management primarily focuses on supportive care, early recognition of warning signs, and prompt intervention to prevent severe complications. Appropriate clinical management significantly reduces mortality, particularly among patients who develop dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS). According to the World Health

Organization (WHO), timely diagnosis combined with careful fluid management can reduce dengue-related mortality to less than 1%.

Management strategies depend on disease severity and are generally categorized into outpatient care, inpatient management, and emergency treatment. Patients with uncomplicated dengue who maintain adequate oral intake and do not exhibit warning signs are managed conservatively with oral rehydration therapy, adequate rest, paracetamol for fever control, and close clinical monitoring. Non-steroidal anti-inflammatory drugs (NSAIDs), including aspirin and ibuprofen, should be avoided because they increase the risk of bleeding.

Patients exhibiting warning signs such as persistent vomiting, severe abdominal pain, mucosal bleeding, lethargy, hepatomegaly, or rising hematocrit require hospitalization for close monitoring and intravenous fluid therapy. Isotonic crystalloids such as normal saline or Ringer's lactate are recommended to maintain adequate intravascular volume while preventing fluid overload. Serial monitoring of hematocrit, platelet count, urine output, blood pressure, and vital signs is essential to guide fluid replacement therapy and detect progression to severe disease.

Patients with severe dengue require intensive care management. Shock should be treated promptly using controlled intravenous crystalloid or colloid solutions according to WHO recommendations. Severe hemorrhage may necessitate blood transfusion or packed red blood cell administration, whereas platelet transfusion is generally reserved for patients with significant bleeding rather than isolated thrombocytopenia. Additional supportive measures include correction of electrolyte imbalance, treatment of metabolic acidosis, oxygen supplementation, and management of organ dysfunction involving the liver, kidneys, or central nervous system.

Recent research has explored antiviral agents, monoclonal antibodies, host-targeted therapies, and immunomodulatory drugs; however, none has yet demonstrated sufficient clinical efficacy for routine practice. Therefore, early diagnosis and supportive treatment remain the cornerstone of dengue management.

8. Prevention and Future Perspectives

Effective dengue prevention requires an integrated strategy involving vector control, environmental management, community participation, laboratory surveillance, and early case detection. Since Aedes aegypti mosquitoes breed primarily in stagnant water collected in artificial containers, eliminating breeding sites remains the most effective preventive measure. Public health authorities encourage regular cleaning of water storage containers, proper waste

disposal, elimination of discarded tires and containers, and covering household water reservoirs to reduce mosquito breeding.

Chemical vector control using larvicides and insecticides continues to play an important role during outbreaks, although increasing insecticide resistance has reduced effectiveness in some regions. Biological control approaches, including the introduction of Wolbachia-infected mosquitoes and genetically modified sterile male mosquitoes, have shown promising results in reducing mosquito populations and interrupting dengue transmission.

Personal protective measures remain equally important. Wearing long-sleeved clothing, using mosquito repellents, installing window screens, sleeping under insecticide-treated nets where appropriate, and minimizing outdoor exposure during peak mosquito activity contribute to reducing mosquito bites and subsequent infection.

Vaccination has emerged as an additional preventive strategy. Dengvaxia® (CYD-TDV) was the first licensed dengue vaccine and is approved for use in selected populations with prior dengue infection. However, because vaccination may increase the risk of severe dengue in seronegative individuals, careful pre-vaccination screening is necessary. Several second-generation vaccines remain under clinical evaluation and may offer broader protection with improved safety profiles.

Future research should focus on identifying reliable biomarkers capable of predicting severe disease during the earliest stages of infection. Viral load measurement using real-time RT-PCR has demonstrated considerable potential in this regard, particularly when combined with hematological and immunological biomarkers. Advances in artificial intelligence, digital health technologies, and portable molecular diagnostic platforms may further improve outbreak surveillance, facilitate rapid diagnosis in resource-limited settings, and optimize patient triage.

Continued investment in public health infrastructure, vector surveillance, molecular diagnostics, and vaccine development will be essential for reducing the global burden of dengue infection.

9. CONCLUSION

Dengue continues to represent a major global public health challenge, particularly in tropical and subtropical countries where favorable environmental conditions facilitate sustained transmission. Despite significant advances in clinical management and laboratory diagnostics, dengue remains responsible for substantial morbidity, mortality, and economic burden. The absence of highly effective antiviral therapy underscores the importance of early diagnosis and supportive management in improving patient outcomes.

Real-time reverse transcription polymerase chain reaction (RT-PCR) has transformed dengue diagnosis by enabling rapid, highly sensitive, and specific detection of viral RNA during the acute phase of infection. Beyond diagnosis, viral load estimation has emerged as an important prognostic biomarker because higher viral RNA concentrations are consistently associated with increased disease severity, plasma leakage, thrombocytopenia, and shock. Quantitative molecular testing therefore assists clinicians in identifying high-risk patients who require intensive monitoring and timely intervention.

Although serological methods remain valuable during later stages of illness, integrating molecular diagnostics with conventional laboratory investigations provides a more comprehensive approach to dengue diagnosis and patient management. Furthermore, multiplex RT-PCR enables simultaneous serotype identification, thereby supporting epidemiological surveillance and outbreak investigations.

Comprehensive dengue control requires coordinated implementation of vector control programs, public education, environmental sanitation, molecular surveillance, vaccination strategies, and continued research into novel antiviral therapies. Strengthening laboratory infrastructure and expanding access to advanced molecular diagnostics will enhance early case detection and improve healthcare responses during outbreaks.

In conclusion, understanding viral load dynamics through real-time RT-PCR provides valuable insights into dengue pathogenesis, disease progression, and prognosis. Continued integration of molecular diagnostics into routine clinical practice will contribute significantly to improved patient outcomes and strengthened dengue control programs worldwide.

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